REPORT

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MAMMALIAN TOXICITY OF MUNITIONS COMPOUNDS
IDENTIFICATION OF WASTE PRODUCTS FROM
RDX AND HMX MANUFACTURE

PROGRESS REPORT NO. 10
April 24, 1979

Contract No. DAMD-17-74-C-4073
Project No. 3900-B-9

For

COTR: Dr. Jack C. Dacre
Technical Monitor: Dr. David H. Rosenblatt
Environmental Protection Research Division
U.S. Army Medical Bioengineering Research and
Development Laboratory
Fort Detrick, Frederick, Maryland 21701

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By

Danny O. Helton
William Burton
Gail Rehagen
Cheng-Chun Lee

Supported by

U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

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Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)
Hexahydro-1-acetyl-3,5-dinitro-1,3,5-triazine (TAX)
Octahydro-1-acetyl-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Wastewaters from the manufacture of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) contain HMX, RDX, hexahydro-1-acetyl-3,5-dinitro-1,3,5-triazine (TAX), octahydro-1-acetyl-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX), cyclohexanone, 2-(1-cyclohexenyl)-cyclohexanone, 2-cyclohexylidenecyclohexanone, 2-cyclohexylcyclohex-2-enone (tentative identification), 2-hydroxymethylcyclohexanone, spiro[1-oxocyclohexane-2,2'-3',4',5',6',7',8'-

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19. 2-(1-cyclohexenyl)-cyclohexanone
2-cyclohexylidenecyclohexanone
2-cyclohexylcyclohex-2-enone
spiro[1-oxocyclohexane-2,2'-3',4',5',6',7',8'-hexahydrobenzo[b]-pyran]
2-hydroxymethylcyclohexanone
cyclohexanone
dimethylnitrosamine
wastewater
munitions

20. hexahydrobenzo(b)pyran. One process unrelated to the direct production of HMX and RDX produces dimethylnitrosamine in a concentration of ∿ 500 ppm and has a flow rate of 43,000 gal/day. This process operates 2 to 3 times per year with a duration of about 1 week each time.

PREFACE

This report was prepared at Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri 64110, under U.S. Department of the Army Contract No. DAMD-17-74-C-4073, Subtask 9, MRI Project No. 3900-B-9, "Identification of Waste Products from RDX and HMX Manufacture." This work was supported by the U.S. Army Medical Bioengineering Research and Development Laboratory, USAMRDC, Department of the Army. Captain John P. Glennon and Dr. Jack C. Dacre, Environmental Protection Research Division, USAMBRDL, were the successive overall technical monitors with Dr. David Rosenblatt serving as technical monitor for this subtask.

This work was performed in the Chemical and Biological Sciences Group under the direction of Dr. Florence I. Metz, Vice President and Executive Director, and Dr. James L. Spigarelli, Director, Analytical Chemistry Department. Experimental work was directed by Dr. Danny O. Helton, Senior Chemist, with assistance from Mr. William Burton, Senior Chemist and Mrs. Gail Rehagen, Assistant Chemist. Dr. Cheng-Chun Lee, Principal Advisor for Pharmacology/Toxicology, or Dr. William House, Director, Biological Sciences Division reviewed all reports.

Approved for:

MIDWEST RESEARCH INSTITUTE

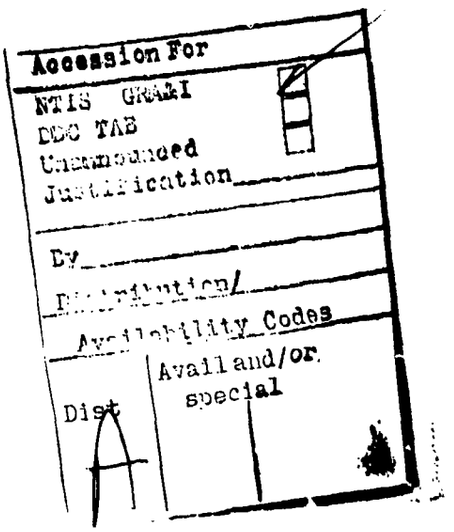
James L. Spigarelli

James L. Spigarelli, Director
Analytical Chemistry Department

for Florence I. Metz

Cheng-Chun Lee, Principal Advisor
for Pharmacology/Toxicology
Pharmacology/Toxicology Department

April 24, 1979



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EXECUTIVE SUMMARY

The objective of this work, authorized by Contract No. DAMD-17-74-C-4073, Subtask 9, was the identification and analysis of organic unknowns in wastewaters resulting from the manufacture of octahydro-1,3,5-trinitro-1,3,5,7-tetrazocine (HMX) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) at the Holston Army Ammunition Plant (HSAAP). This work was conducted to aid in identifying potentially toxic and/or carcinogenic compounds with significant concentrations in the wastestreams.

Three groups of samples were studied: (a) nitramine components; (b) cyclohexanone-related components; and (c) samples containing dimethylnitrosamine. The nitramine work required the use of field ionization mass spectrometry (FI/MS), high performance liquid chromatography (HPLC), and infrared spectroscopy. Reference samples for the nitramine work were supplied by Holston Army Ammunition Plant (HSAAP). The work with cyclohexanone-related components utilized gas chromatography/mass spectrometry (GC/MS). MRI synthesized several samples for positive identification and supplied these to HSAAP for use in routine monitoring. The dimethylnitrosamine work utilized GC/MS and GC with an alkali flame ionization detector.

The following nitramines were identified in the wastewaters:

Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX),
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX),
Hexahydro-1-acetyl-3,5-dinitro-1,3,5-triazine (TAX),
Octahydro-1-acetyl-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX).

The following cyclohexanone related components were identified:

2-(1-cyclohexenyl)-cyclohexanone,
2-cyclohexylidenecyclohexanone,
2-cyclohexylcyclohex-2-enone (tentative identification),
2-hydroxymethylcyclohexanone,
Spiro[1-oxocyclohexane-2,2'-3',4',5',6',7',8'-hexahydrobenzo [b]pyran].

Dimethylnitrosamine (DMN) was found in the wastewater from HSAAP building A-1 at a concentration of approximately 500 ppm. Since the time from wastewater sampling to analysis was about 1 month, DMN formation during storage must be considered. The samples were stored at 4°C at pH 6-7. Nitrosamine formation under these circumstances is expected to be very slow.

I. INTRODUCTION

Under Contract No. DAMD-17-74-C-4073, Subtask 9, entitled "Identification of Waste Products from RDX and HMX Manufacture," three groups of wastewater samples from Holston Army Ammunition Plant (HSAAP), Kingsport, Tennessee, have been analyzed. This work was authorized to aid in identifying and assaying potentially toxic compounds in HSAAP wastestreams.

Techniques used in this work include field ionization mass spectrometry (FI/MS), infrared spectrometry, capillary gas chromatography, and high performance liquid chromatography (HPLC). Details of most of the work has been reported in monthly progress reports. Several of these reports are included to provide experimental details.

In the following sections are discussed Identification of Wastewater Components Isolated by HSAAP (II), Identification of Cyclohexanone-Related Components Isolated by Midwest Research Institute (III), Identification and Quantitation of Dimethylnitrosamine in Wastestreams (IV), and Conclusions (V).

II. IDENTIFICATION OF WASTEWATER COMPONENTS ISOLATED BY HSAAP

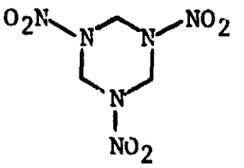
To aid identification work, a compilation of potentially present nitramines and related compounds was made (see Appendix A). This compilation is arranged by molecular weight and provides compound structure and common names.

Appendix B summarizes work in this area and discusses sample origin, identification procedures, percentage of unknowns identified, suggested procedures for routine monitoring, and the source of compounds for potential toxicological or additional chemical studies.

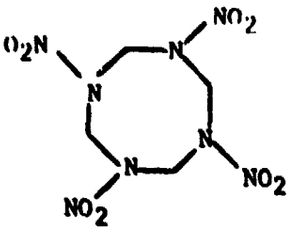
Field ionization mass spectrometry of these nitramines has not been previously reported in the open literature. These spectra are included in Appendix C.

From the samples submitted for identification, four different compounds were identified. These were:

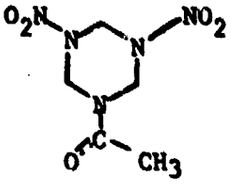
1. Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)



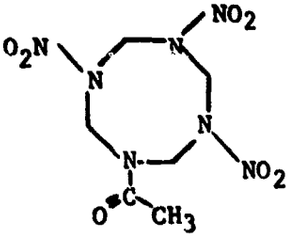
2. Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)



3. Hexahydro-1-acetyl-3,5-dinitro-1,3,5-triazine (TAX)



4. Octahydro-1-acetyl-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX)



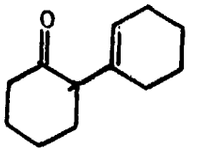
All other components were too impure for identification. HSAAP indicated methylene dinitrate was present by HPLC retention time; however, MRI has not been able to confirm this.

Reference samples of the identified nitramines were available at HSAAP for use in routine monitoring.

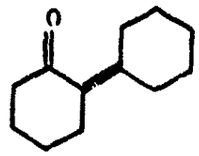
III. IDENTIFICATION OF CYCLOHEXANONE-RELATED COMPONENTS ISOLATED BY MIDWEST RESEARCH INSTITUTE

Appendix D summarizes work in this area. Compounds identified include:

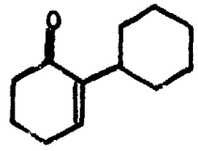
1. 2-(1-Cyclohexenyl)-cyclohexanone



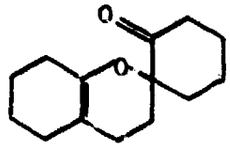
2. 2-Cyclohexylidenecyclohexanone



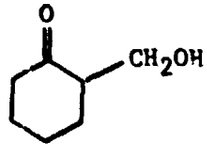
3. 2-Cyclohexylcyclohex-2-enone (tentative identification only)



4. Spiro[1-oxocyclohexane-2,2'-3',4', 5',6',7',8'-hexahydrobenzo[b]pyran]



5. 2-Hydroxymethylcyclohexanone



Reference samples of these materials have been shipped to HSAAP for use in routine monitoring.

IV. IDENTIFICATION AND QUANTITATION OF DIMETHYLNITROSAMINE IN WASTEWATER

Appendix E summarizes work in this area.

The presence of dimethylnitrosamine (DMN) was confirmed by two independent techniques, gas chromatography using an alkali flame ionization detector (GC/AFID) and capillary gas chromatography/mass spectrometry. The DMN concentratons determined by GC/AFID ranged from 1 to ∿ 500 ppm (parts per million).

Since DMN was found in high concentrations the likelihood of formation during storage, about 1 month, must be considered. A large amount of information on nitrosation of secondary amines is available.[1,2]

The optimum pH for nitrosation of dimethylamine is 3.4[3] whereas the pH of the wastewater samples at Sites 1, 2, and 3 were 7.8, 6.2, and 6.8, respectively. The nitrosation rate for strongly basic amines is much less than that of weakly basic amines such as piperazine (~ 50,000:1).[3] A basic secondary amine, such as diethylamine, requires high concentrations of nitrite of the order of 0.1 M and 37° for 3 hr at pH 1.0 to 3.4 to be even 5% nitrosated.[4] Based on the reported dependence of nitrosamine formation on pH[5] the rate of formation above pH 6 should be very low, in fact no data was found on nitrosation of strongly basic amines above pH 6.

Most reported data on nitrosamine formation has been conducted at 37°. No data were found for the actual sample storage temperature of 4°. Bacterial production of DMN is also expected to be low under these storage conditions.[6]

V. CONCLUSIONS

The major nitramine components in wastewater from manufacture of RDX and HMX at HSAAP are RDX, HMX, TAX, and SEX. The major cyclohexanone related components in these wastewaters are 2-(1-cyclohexenyl)-cyclohexanone, 2-cyclohexylidenecyclohexanone, 2-cyclohexylcyclohex-2-enone (tentative identification) and spiro[1-oxocyclohexane-2,2'-3',4',-5',6',7',8'-hexahydrobenzo[b]pyran]. Dimethylnitrosamine occurred in a

1/ N-Nitroso Compounds Analysis and Formation, Ed. by P. Bogouski, R. Preussmann, E. A. Walker, and W. Davis, International Agency for Research on Cancer, World Health Organization, 1972.

2/ N-Nitroso Compounds in the Environmenta, Ed. by P. Bogouski, E. A. Walker, and W. Davis, International Agency for Research on Cancer, World Health Organization, 1974.

3/ N-Nitroso Compounds Analysis and Formation, Ed. by P. Bogouski, R. Preussmann, E. A. Walker, and W. Davis, International Agency for Research on Cancer, World Health Organization. p. 104-105, 1972.

4/ N-Nitroso Compounds Analysis and Formation, Ed. by P. Bogouski, R. Preussmann, E. A. Walker, and W. Davis, International Agency for Research on Cancer, World Health Organization, p. 122, 1972.

5/ N-Nitroso Compounds in the Environmenta, Ed. by P. Bogouski, E. A. Walker, and W. Davis, International Agency for Research on Cancer, World Health Organization, p. 137, 1974.

6/ N-Nitroso Compounds Analysis and Formation, Ed. by P. Bogouski, R. Preussmann, E. A. Walker, and W. Davis, International Agency for Research on Cancer, World Health Organization, p. 116, 1972.

concentration of ∿ 500 parts per million in the wastewater from Building A-1. Building A-1 is not used directly in the manufacture of RDX and HMX.

For most of the above compounds reference samples are now available at HSAAP for use in routine monitoring.

A review of literature on nitrosamine formation suggests a low probability for formation of significnat concentrations of DMN under sample storage conditions; however, this possibility cannot be entirely excluded.

APPENDIX A

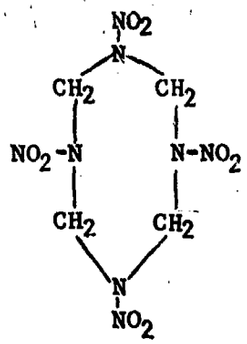
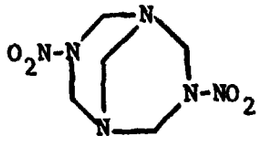
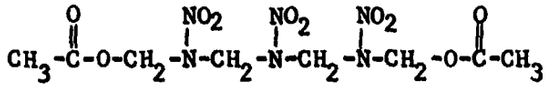
POTENTIALLY PRESENT NITRAMINES AND COMPOUNDS RELATED TO RELATED NITRAMINES

APPENDIX A

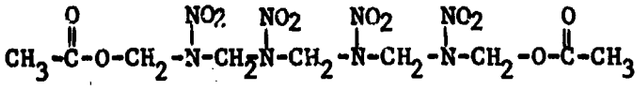
I. Reported Nitramines Arranged by Molecular Weight

Molecular Weight	Sample No.
434.25	020
428.33	004
425.37	030
360.2	017
354.38	034
354.28	003
340.3	021
337.3	015
323.27	011
298.26	019
296.24	006
296.2	001
295.26	032
293.24	010
292.37	034-5
288.36	023
284.36	026
282.26	008
280.29	035
274.33	022
268.23	009
233.25	032-5
226.35	023-5
219.19	016
218.28	035-5
218.21	002
217.27	031
214.25	028
212.32	022-5
212.29	024
178.13	005
115.26	031-5
128.15	025

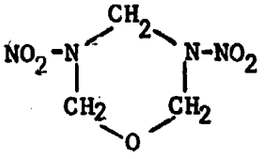
II. Reported Nitramines Arranged by Sample No.

Sample No.	Formula	MW	Structure
001 (HMX)	$C_4H_8N_8O_8$	296.2	
002 (DNPT or DPT)	$C_5H_{10}N_4O_6$	218.21	
003 (BSX)	$C_8H_{14}N_6O_{10}$	354.28	

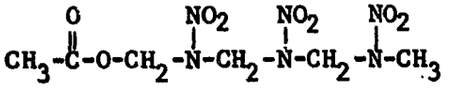
Sample No.	Formula	MW	Structure
004 AcAn	$C_9H_{16}N_8O_{12}$	428.33	



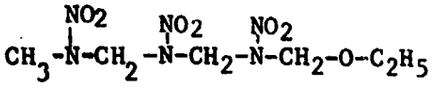
005 Cyclonite Oxide	$C_3H_6N_4O_5$	178.13	



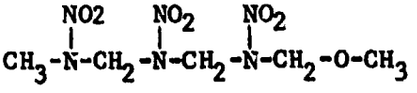
C06 MSX	$C_6H_{12}N_6O_8$	296.24	

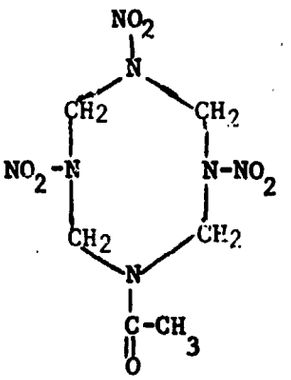
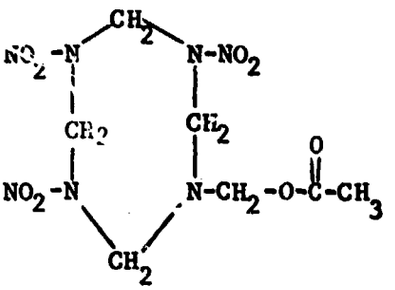
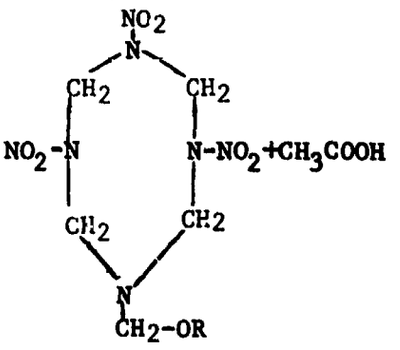


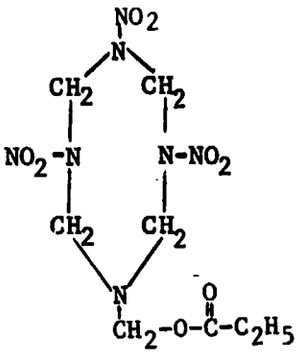
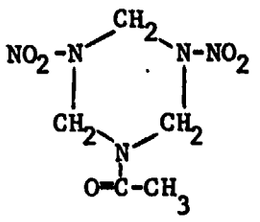
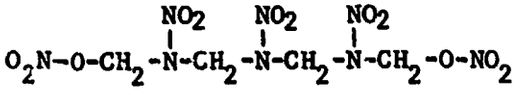
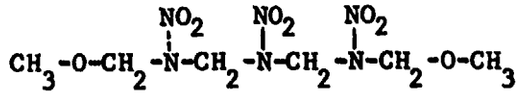
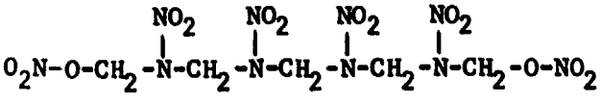
007 CMX	Unknown (from a thesis by G. C. Bassler, "The Chemistry of Cyclonite," Pennsylvania State College, 1943)		
008 H-24	$C_6H_{14}N_6O_7$	282.26	

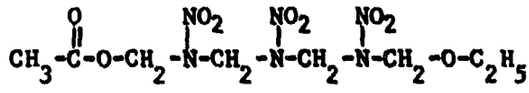
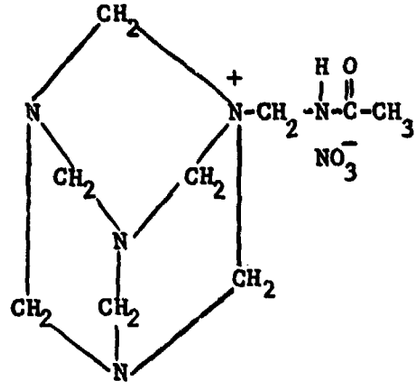


009 H-25	$C_5H_{12}N_6O_7$	268.23	

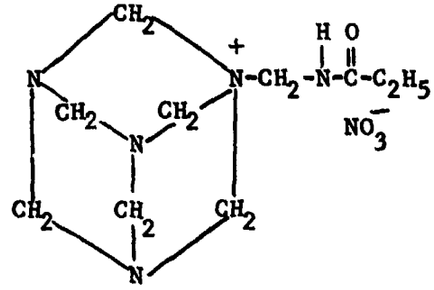


Sample No.	Formula	MW	Structure
010 SEX or QDX	$C_6H_{11}N_7O_7$	293.24	
011 (PHX)	$C_7H_{13}N_7O_8$	323.27	
012 Me-HX	R = CH_3		
013 Et-HX	R = C_2H_5		
014 Butyl-HX	R = C_4H_9		

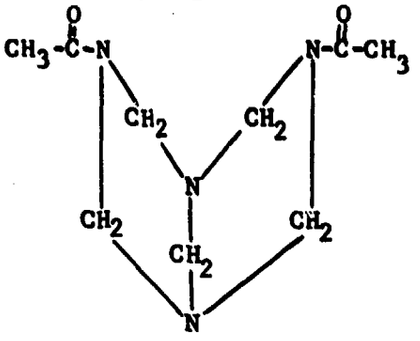
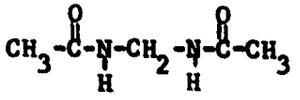
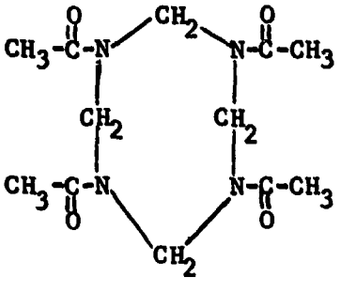
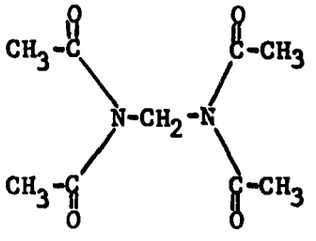
Sample No.	Formula	MW	Structure
015 p^2HX	$C_8H_{15}N_7O_8$	337.3	
016 TAX	$C_5H_9N_5O_5$	219.19	
017 ATX	$C_4H_8N_8O_{12}$	360.2	
018 PSI	Unknown (from a thesis by G. C. Bassler, "The Chemistry of Cyclonite," Pennsylvania State College, 1943)		
019 "104"	$C_6H_{14}N_6O_8$	298.26	
020 106	$C_5H_{10}N_{10}O_{10}$	434.25	

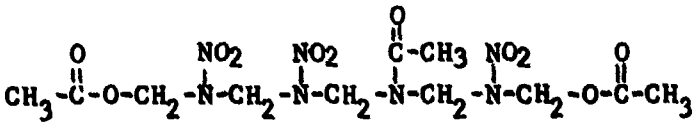
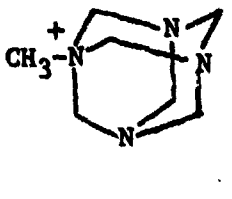
Sample No.	Formula	MW	Structure
021 (107)	$C_8H_{16}N_6O_9$	340.3	
022 (H-2)	$C_9H_{18}N_6O_4$	274.33	

MW of organic group is 212.32

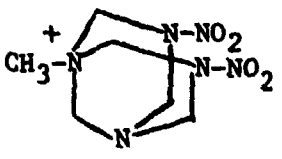
023 (H-5)	$C_{10}H_{20}N_6O_4$	288.36	

MW of organic group is 226.35

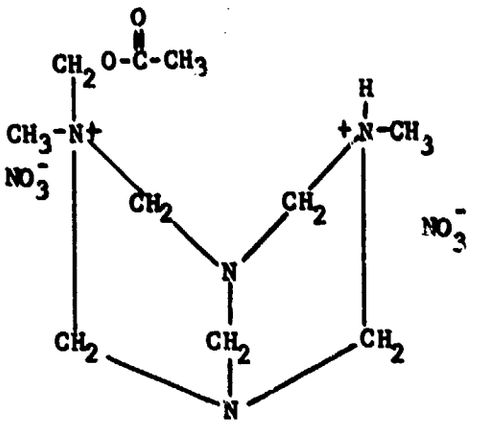
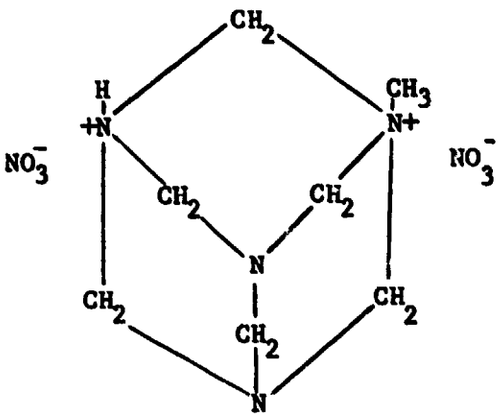
Sample No.	Formula	MW	Structure
024 H-6 (DAPT)	$C_9H_{16}N_4O_2$	212.29	
025 H-7	$C_5H_8N_2O_2$	128.15	
026 H-8	$C_{12}H_{20}N_4O_5$	284.36	
027 H-9	Unknown		
028 H-10	$C_9H_{14}N_2O_4$	214.25	

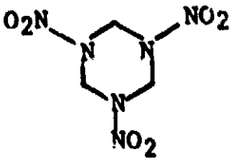
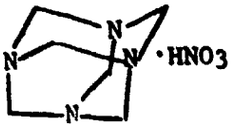
Sample No.	Formula	MW	Structure
029 H-11	Unknown (from a thesis by G. C. Bassler, "The Chemistry of Cyclonite," Pennsylvania State College, 1943)		
030 H-16	$C_{11}H_{19}N_7O_{11}$	425.37	
031 H-18	$C_7H_{15}N_5O_3$	217.27	 NO_3^-

MW of organic group is 155.26

032 H-19	$C_6H_{13}N_7O_7$	295.26	 NO_3^-

MW of organic group is 233.25

Sample No.	Formula	MW	Structure
033 H-22	Not available		
034 H-23	$C_{10}H_{22}N_6O_8$	354.38	 MW of organic group is 292.37
035 H-26	$C_7H_{16}N_6O_6$	280.29	 MW of organic group is 218.28

Sample No.	Formula	MW	Structure
036 TTP	$C_2H_6N_6O_6$	210.12	
037 RDX	$C_3H_6N_6O_6$	222.13	
038 HAMN	$C_6H_{13}N_5O_3$		

APPENDIX B

IDENTIFICATION OF NITRAMINES

November 9, 1977

Major Illar Muul
Attn: SGRD-UBG
Environmental Protection Department
U.S. Army Medical Bioengineering
Research and Development Laboratory
Fort Detrick
Frederick, Maryland 21701

Subject: Subtask 9, Identification of Waste Products from RDX and HMX Manufacture, Monthly Report No. 15.

Name of Contractor: Midwest Research Institute
425 Volker Boulevard
Kansas City, Missouri 64110

Program Director: Dr. Cheng-Chun Lee

Phone Number: 816-753-7600

Date of Report: November 9, 1977

Period Covered: July 1 through September 30, 1977

Gentlemen:

All the "purified" fractions from the RDX Line and HMX Line have been identified or demonstrated to be too impure for identification without further chromatographic purification. Appendix A summarizes results on the RDX Line samples. Appendix B discusses individual RDX Line samples which were partially characterized or are too impure for identification. Appendix C summarizes results on the HMX Line samples. Appendix D discusses individual HMX Line samples which were partially characterized or too impure for identification. Appendix E discusses mass spectra of the reference compounds.

Identification of impurities in the cyclohexanone being dumped in the river has been resumed. This work was halted from the last part of July until August 4 when a more appropriate sample arrived from Holston AAP. This sample is being studied by HPLC for UV absorbing components, and capillary GC-MS. HPLC indicates numerous UV absorbing components.

Major Illar Muul
U.S. Army Medical Bioengineering
Research and Development Laboratory

RDX Line Unknowns

A. Sample Origin

Samples received by MRI were purified at Holston AAP (HAAP). The following purification scheme was provided by HAAP:

About 60 liters of water taken from the RDX "dewater" process were extracted with a mixture of dichloromethane and acetonitrile. The extracts were combined and the solvent removed by distillation at atmospheric pressure until a volume of about 150 ml was left in the still pot. The remaining solvent was removed by vacuum distillation. Solids remaining after evaporation of the extraction solvent were separated by liquid chromatography into six major fractions. The six fractions were then refractionated into 14 components. Operating conditions for the liquid chromatograph for the initial fractionation and for the refractionation are attached with the respective LC scans.

A total of 18 samples were received for identification, plus 16 reference samples. An HPLC chromatogram of the initial fractionation using a preparative LC column is given in Figure 1. Figure 2 shows a chromatogram with peak identifications of the same material using an analytical grade LC column.

The samples were shipped to Midwest Research Institute unrefrigerated, then stored refrigerated until used.

B. Identification Procedures

Sample identification has been by one or more of the following techniques: mass spectrum, infrared spectrum, and high pressure liquid chromatography retention time. Samples which could not be identified by mass spectrum and/or infrared spectrum were studied by HPLC to determine sample purity. In a few cases where mass spectral and infrared spectra gave equivocal identifications, subsequent study by HPLC allowed identification of the major component.

To provide a field ionization mass spectral data base for use in interpretation of unknowns, 16 reference samples were examined. Of those examined, 12 gave interpretable spectra (see Appendix E).

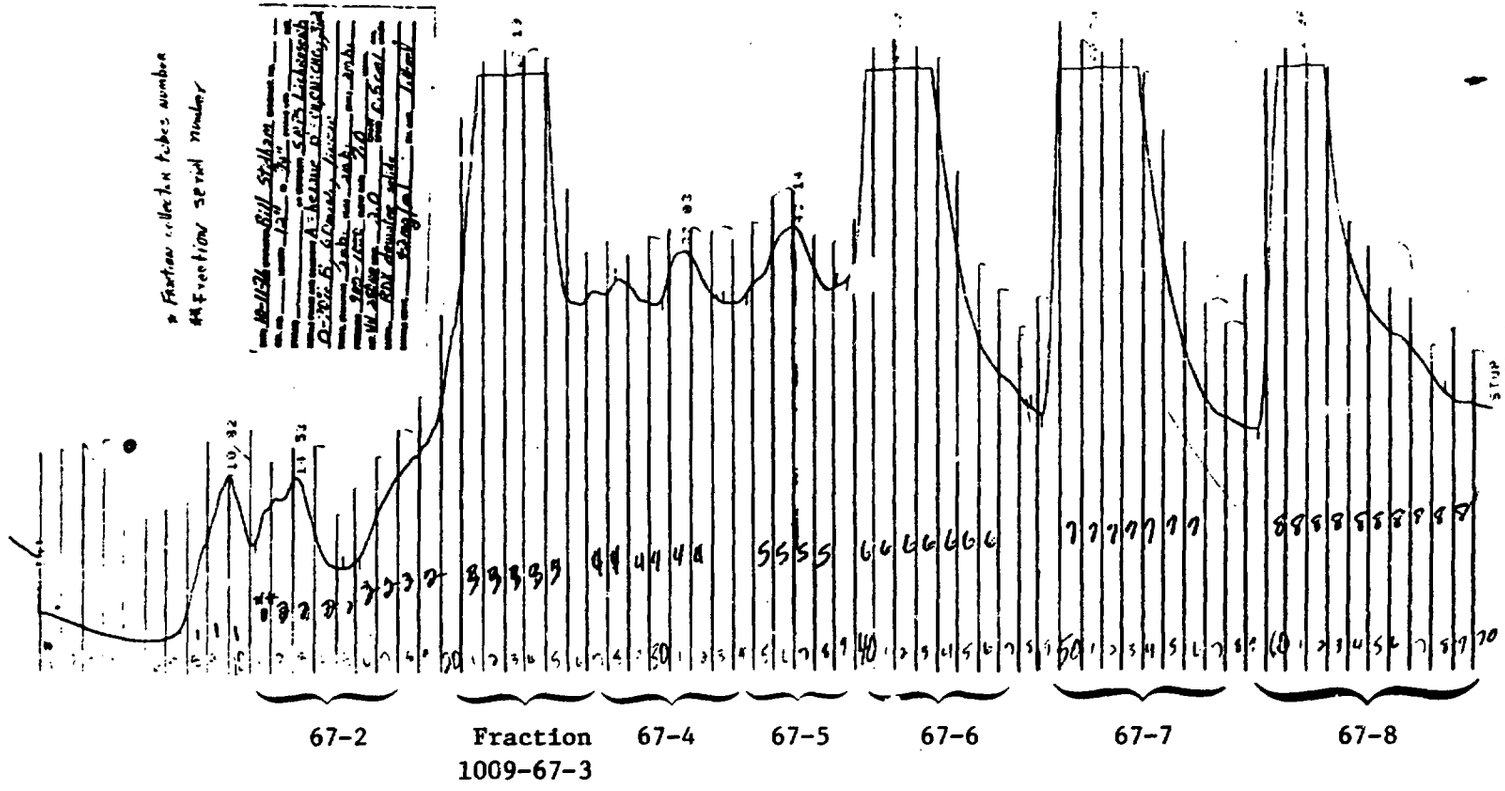


Figure 1 - HPLC Chromatogram of RDX Line Unknowns Using Oreparatine LC Column

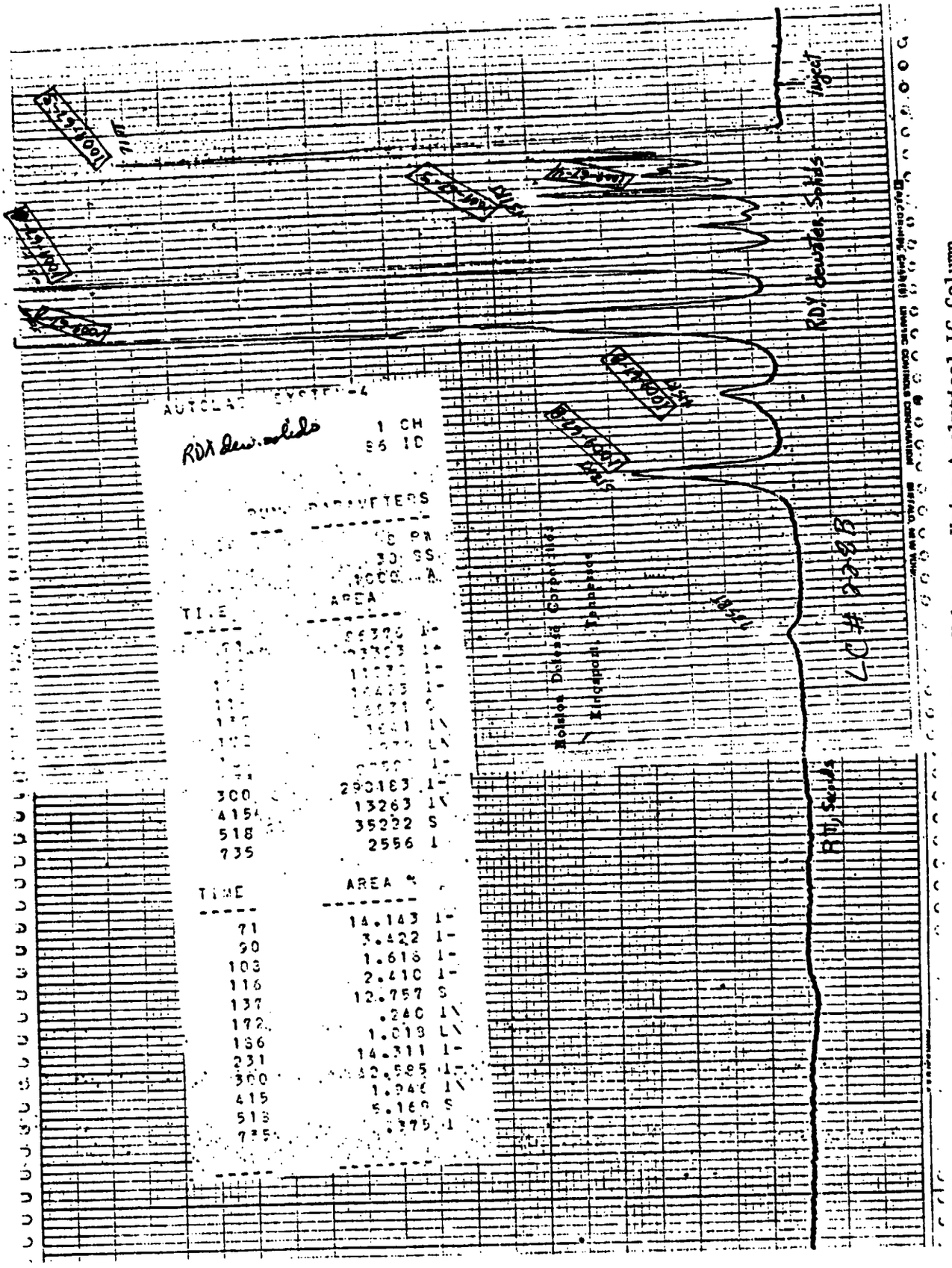


Figure 2 - HPLC Chromatogram of RDX Line Unknowns Using Analytical LC Column

C. Percentage of Unknowns Identified

Review of the sample preparation procedures indicates two areas which make quantitation of the identified components difficult without additional data. The first area concerns compounds which may not have been extracted using dichloromethane and acetonitrile, specifically compounds which are ionic such as H-2, H-18, H-19, H-23, H-26, PCX, and methylamine nitrate (structures are given in Report No. 10). Compounds which would be extracted but may be lost during workup include nitromethane and methylnitrate. The second difficulty is that although Holston AAP has provided a HPLC chromatogram of the RDX Line unknowns and peak area integration of the components, the individual sample weights are unknown. Inferring actual percent concentrations from the peak area data can introduce significant error as judged by the Holston AAP HPLC UV detector setting of 280 nm and the following spectral data:

Compound in Ethanol	λ_{max}[1/]	ϵ_{max}[1/]
RDX	213	11,000
HMX	228	21,000
TAX	231-234	6,500
SEX	227	15,800
Cyclohexanone	284	18
2-Cyclohexylidenecyclohexanone	256	6,460[2/]

1/ Jones, R. N., and G. D. Thorn, Can J. Research, 27B, 828 (1949).
2/ Horak, M., and P. Munk, Coll. Czech. Chem. Comm., 24, 3024 (1959).

The data gathered suggest the concentrations of RDX, HMX, TAX, SEX, and methylene dinitrate account for more than 50% of the organics extracted. An HPLC assay of the extract using standards of each of the known components would yield more precise information. This work could most easily be accomplished at Holston AAP.

D. Suggested Procedures for Routine Monitoring

The extraction and HPLC work by Holston AAP seems adequate for monitoring of all the identified nitramines (see A. Sample Origin). Aqueous or alcoholic solutions of nitramines have an absorption maxima in the region 225 to 240 nm. For monitoring these compounds a UV detector set at 230 nm is recommended.

Identification of the cyclohexanone related components is in progress and recommendations for monitoring will be made later. Gas chromatography using a flame ionization detector may well be more suitable than HPLC for monitoring some cyclohexanone derivatives due to lack of a significant UV chromaphore. Previously identified cyclohexenylcyclohexanone is an exception in that it has a strong UV chromaphore; however, it too is readily assayed by GC.

E. Source of Compounds for Potential Toxicological or Additional Chemical Studies

Three groups have expressed interest in preparing moderately large quantities ($\sim$150 g each) of nitramines to be used for toxicology work. These are Holston AAP (Russell Jackson, 615-247-9111), University of Texas at San Antonio (Budalur S. Thyagarajan, Division of Earth and Physical Sciences,

San Antonio, Texas 78285, 512-691-4455), and Dr. Irwin J. Solomon (IITRI, 10 West 35th Street, Chicago, Illinois 60616, 312-567-4000).

Sincerely,

MIDWEST RESEARCH INSTITUTE

Dan Helton

Danny O. Helton
Senior Chemist

Approved:

W. B. House, Director
Biological Sciences Division

(15 copies of report submitted)

cc: Ms. Jean Smith
Contract Office
U.S. Army Research and Development Command
Washington, D.C. 20314

Dr. David Rosenblatt
Environmental Quality Division
U.S. Army Medical Bioengineering Research and Development Laboratory
Fort Detrick
Frederick, Maryland 21701

Mr. Russell Jackson
Holston Army Ammunition Plant
Kingsport, Tennessee 37662

MONTHLY REPORT NO. 15

APPENDIX A

SAMPLE IDENTIFICATION STATUS ON
RDX LINE UNKNOWNS

RDX Line Unknowns

No.	Holston AAP Sample Code	Identification Status		
		HPLC	Infrared Spectrum	Mass Spectrum
1	1009-67-3 IR 4974	Mixture, a	b No NNO_2 bonds	b No NNO_2 bonds
2	1009-67-4 RDX "dewater solid" Fraction No. 4	Mixture, a	c	b, a
3	1009-67-5 RDX "dewater" solid Fraction No. 5	Mixture, a	c	Mixture, a
4	1009-67-6	RDX	RDX	c
5	1009-67-6(7) $CHCl_3$ solubles	RDX is major component	RDX	Mixture of RDX, TAX, and unknown
6	1009-67-6 CCl_4 solubles	Mixture, a	b	Mixture, a
7	1009-67-6 CCl_4 insolubles	Mixture	b	Mixture, contains RDX
8	1009-67-7 IR4982	TAX	TAX	TAX
9	1009-67-7 IR4983	c	TAX	TAX
10	1009-67-7 IR4973	TAX is major component	Mixture	Mixture
11	1009-67-7 IR4967	TAX	TAX	TAX
12	1009-67-7 7X	TAX is major component	Mixture	c
13	1009-67-7 IR4981, $CHCl_3$ A	c	TAX	TAX

RDX Line Unknowns (cont.)

No.	Holston AAP Sample Code	Identification Status HPLC	Infrared Spectrum	Mass Spectrum
14	1009-67-7	Methylene dinitrate and TAX	c	Mixture containing TAX
15	1009-67-7 Fraction 2	TAX	c	TAX
16	1009-67-8 IR4977	c	SEX	SEX
17	1009-67-8 IR4975 $CHCl_3$ solubles	SEX	SEX	SEX
18	1009-67-8 IR4979 $CHCl_3$ solubles	SEX	SEX	SEX

a/ Too impure for identification.
b/ Studied but not identified.
c/ Not studied.
d/ Identification by Holston AAP.

RDX Line Unknowns

No.	Holston AAP Sample Code	Identification Status		
		HPLC	Infrared Spectrum	Mass Spectrum
1	1009-67-3 IR 4974	Mixture, a	b No NNO_2 bonds	b No NNO_2 bonds
2	1009-67-4 RDX "dewater solid" Fraction No. 4	Mixture, a	c	b, a
3	1009-67-5 RDX "dewater" solid Fraction No. 5	Mixture, a	c	Mixture, a
4	1009-67-6	RDX	RDX	c
5	1009-67-6(7) $CHCl_3$ solubles	RDX is major component	RDX	Mixture of RDX, TAX, and unknown
6	1009-67-6 CCl_4 solubles	Mixture, a	b	Mixture, a
7	1009-67-6 CCl_4 insolubles	Mixture,	b	Mixture, contains RDX
8	1009-67-7 IR4982	TAX	TAX	TAX
9	1009-67-7 IR4983	c	TAX	TAX
10	1009-67-7 IR4973	TAX is major component	Mixture	Mixture
11	1009-67-7 IR4967	TAX	TAX	TAX
12	1009-67-7 7X	TAX is major component	Mixture	c
13	1009-67-7 IR4981, $CHCl_3$ A	c	TAX	TAX

RDX Line Unknowns (cont.)

No.	Holston AAP Sample Code	Identification Status		
		HPLC	Infrared Spectrum	Mass Spectrum
14	1009-67-7 Fraction 1	Methylene dinitrate and TAX d/	c	Mixture containing TAX
15	1009-67-7 Fraction 2	TAX	c	TAX
16	1009-67-8 IR4977	c	SEX	SEX
17	1009-67-8 IR4975 $CHCl_3$ solubles	SEX	SEX	SEX
18	1009-67-8 IR4979 $CHCl_3$ solubles	SEX	SEX	SEX

a/ Too impure for identification.
b/ Studied but not identified.
c/ Not studied.
d/ Identification by Holston AAP.

MONTHLY REPORT NO. 15

APPENDIX B

DISCUSSION OF RDX LINE SAMPLES
WHICH WERE PATIALLY CHARACTERIZED

Several samples could not be interpreted by studying their infrared and mass spectras. These samples were examined using the following HPLC system:

1. Instrument: Waters Associates.
2. Column: 30 cm x 4 mm I.D., μBondapak C_{18}.
3. Soluent: Linear program from 100% water to 100% methanol in 20 min.
4. Flow Rate: 1 ml/min.
5. Detector: UV at 254 nm.
6. Results: The results for the individual samples are given below.

Sample 1009-67-3, IR4974

HPLC indicated numerous components (Figure 3). The IR is similar to that of cyclohexenylcyclohexanone and mass spectra indicate an absence of nitramines based no significant m/e 46 (NO_2) peak.

This sample probably contains a mixture of cyclohexanone related components. The sample will be studied by GC-MS for further characterization.

Sample 1009-67-4, RDX "dewater solid" Fraction No 4

HPLC (Figure 4) indicates numerous components in significant relative concentration. This sample is spectrally similar to sample 1009-67-3.

Sample 1009-67-5, RDX "dewater solid" Fraction No 5

HPLC (Figure 5) indicates a mixture. The mass spectrum also suggested a mixture, but unlike the above sample an appreciable m/e 46 peak was observed, indicating the presence of a nitramine.

Sample 1009-67-6, CCl_4 Solubles, IR4946

HPLC (Figure 6) indicates numerous components and is described in the Holston AAP data package as a viscous red/brown semi-solid. Mass spectral data also suggests a mixture. The presence of a prominent m/e 46 indicates the presence of NNO_2. IR suggests the sample is primarly composed of cyclohexanone related components although nitramines are present as indicated by an absorption at 3040 cm^{-1}.

Sample 1009-67-6, CCl_4 Insolubles, IR4943

HPLC (Figure 7) indicates numerous components. IR indicates strong absorptions characteristic of RDX and cyclohexanone derivatives. Mass spectra were inconclusive except to indicate the presence of nitramines.

Sample 1009-67-7, IR4973

By HPLC (Figure 8) the major component is TAX.

Sample 1009-67-7, IR4967

IR, HPLC (Figure 9) and mass spectrum indicate the presence of TAX.

Sample 1009-67-7, 7X

HPLC (Figure 10) indicated one major component and several minor ones. By HPLC retention time the major component is TAX. The IR spectrum also indicated the presence of TAX.

Sample 1009-67-7, Fraction 1

HPLC (Figure 11) indicated one major component (~50%) and several minor components. By retention time the major component is TAX and this is confirmed by mass spectrum. Holston AAP stated that methylene dinitrate was also present as judged by HPLC retention time. MRI could not confirm the presence of methylene dinitrate due to lack of a reference sample and the presence of several additional components in this sample.

Blank HPLC Run

Figure 12 is a blank HPLC run showing instrument drift at longer retention time.

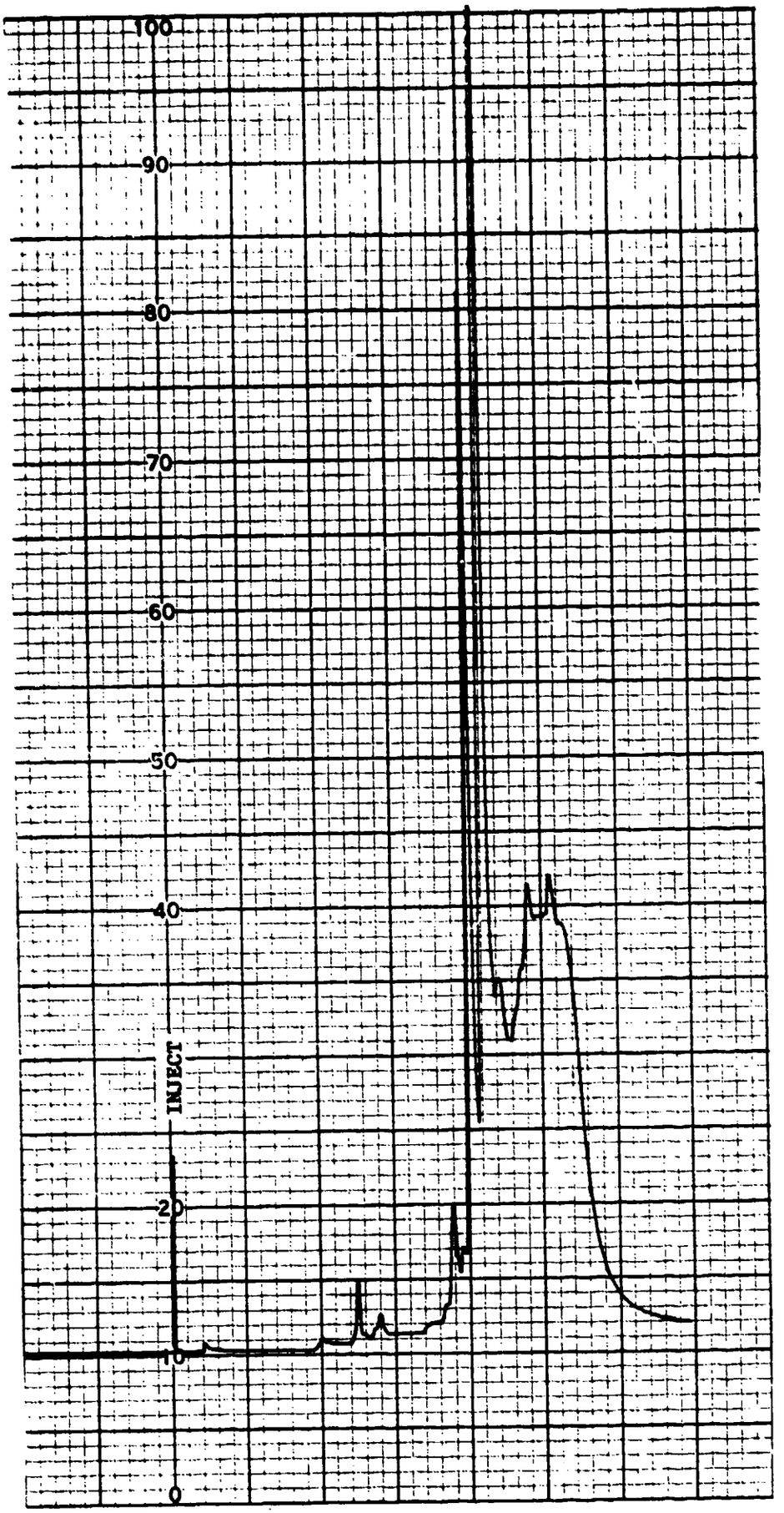


Figure 3 - 1009-67-3, IR4974

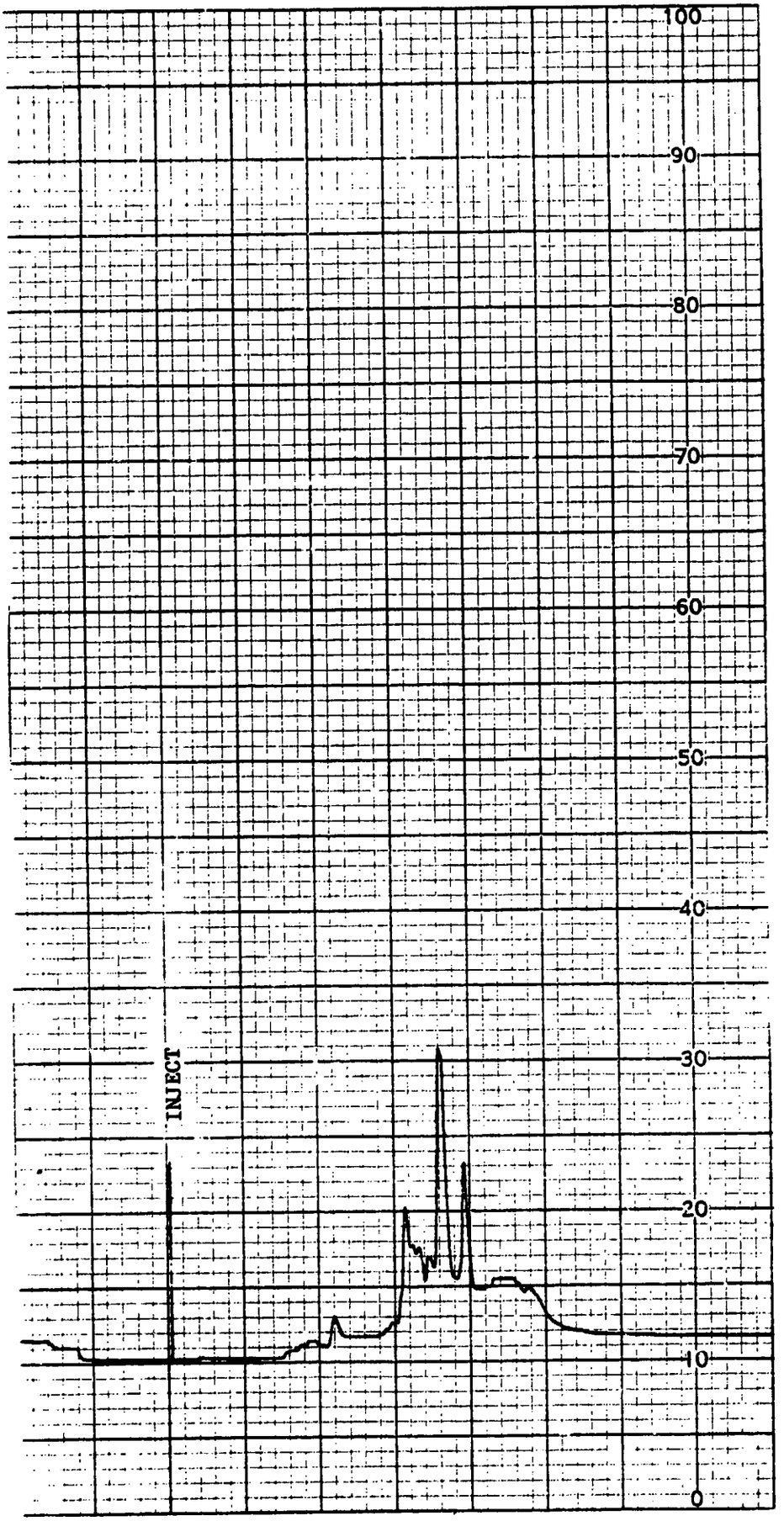


Figure 4 - 1009-67-4

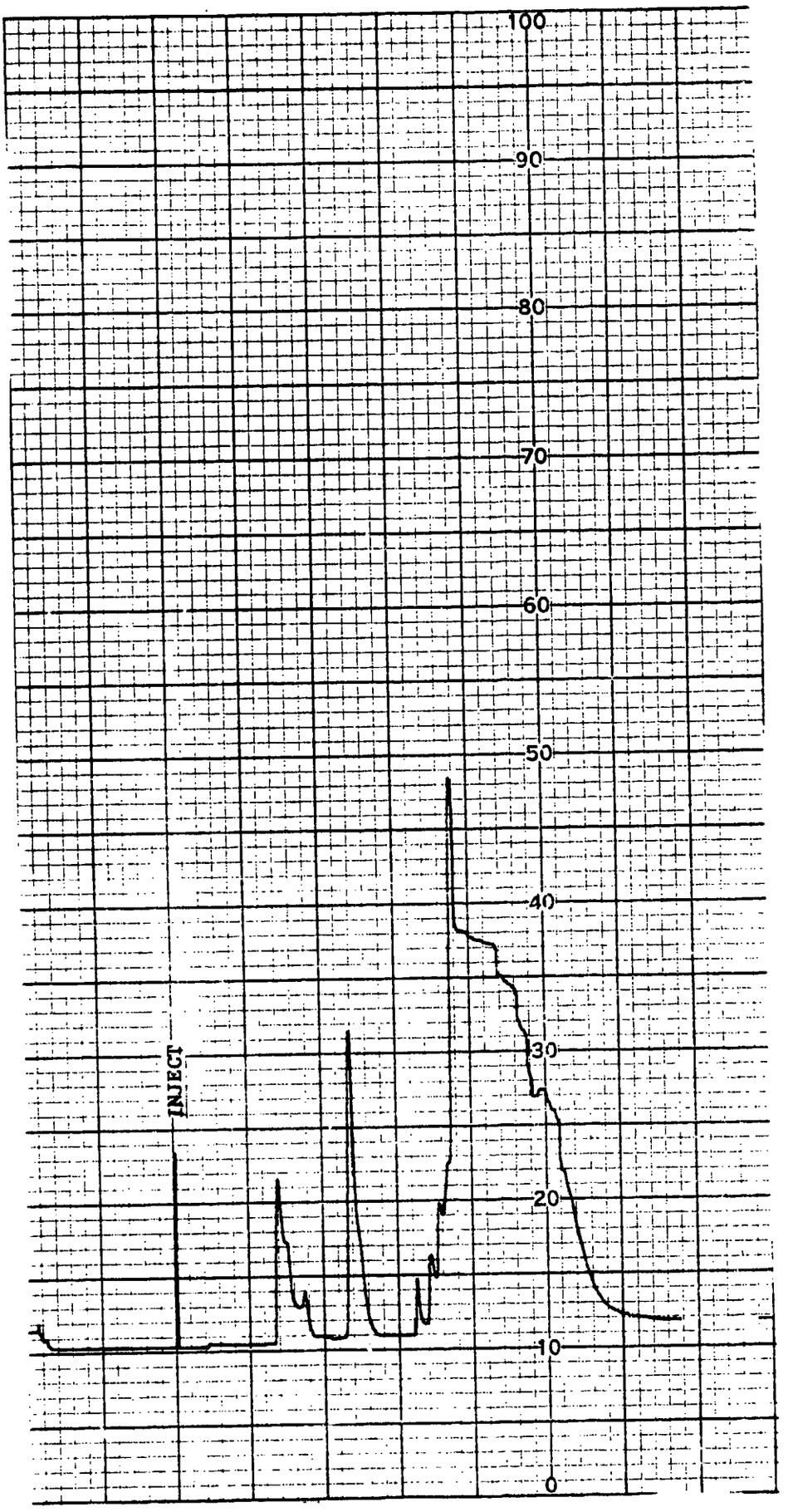


Figure 5 - 1009-67-5

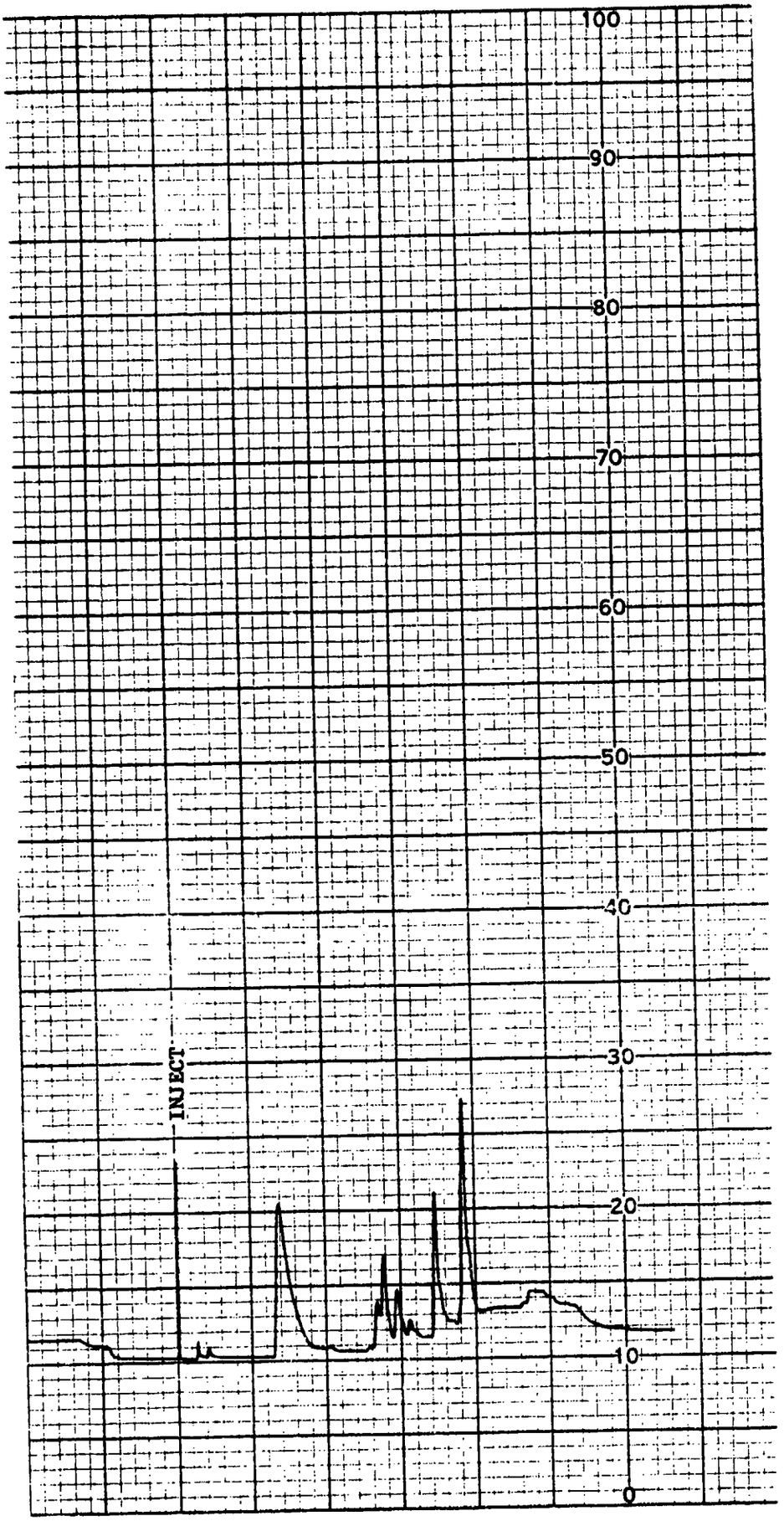


Figure 6 - 1009-67-6, CCl_4 Solubles

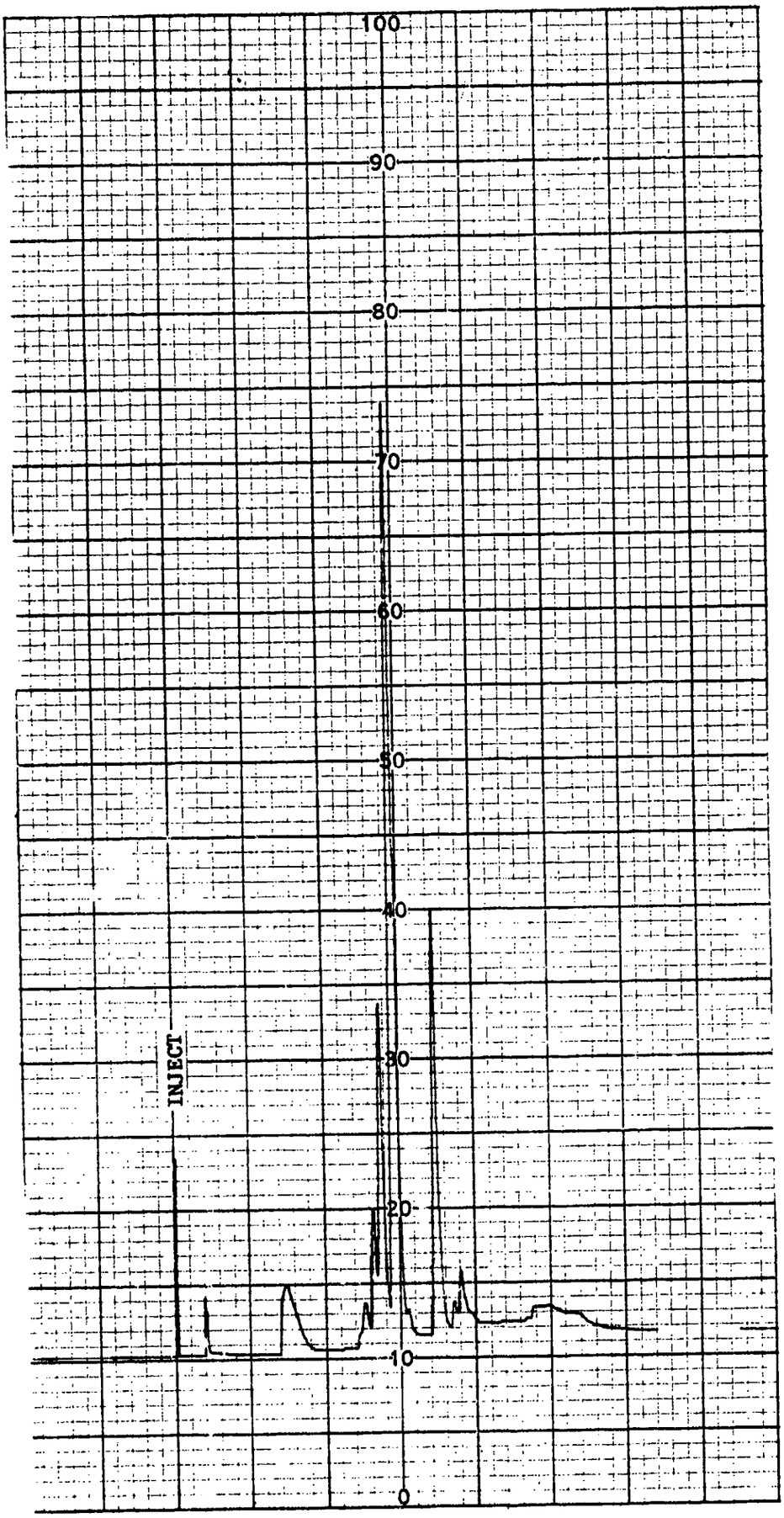


Figure 7 - 1009-67-6, CCl_4 Insolubles, IR4973

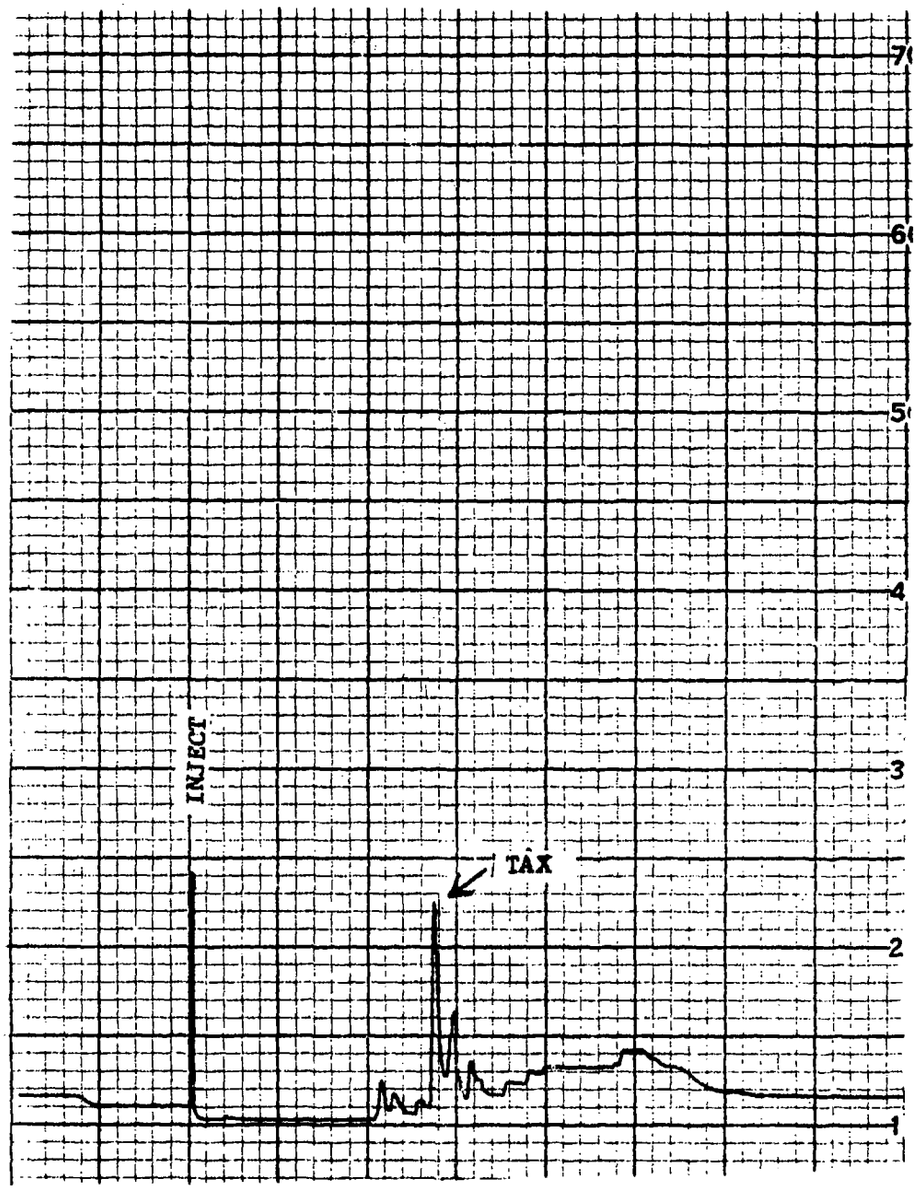


Figure 8 - 1009-67-7, IR4973

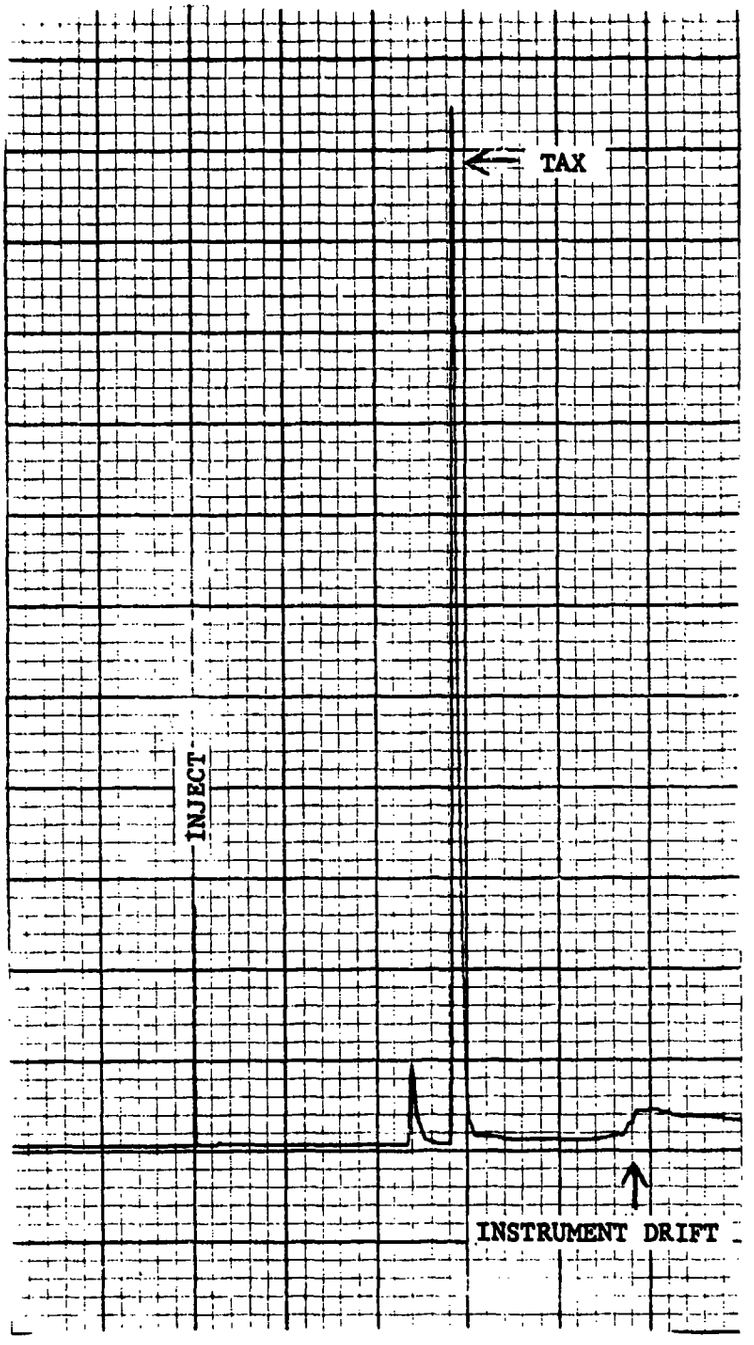


Figure 9

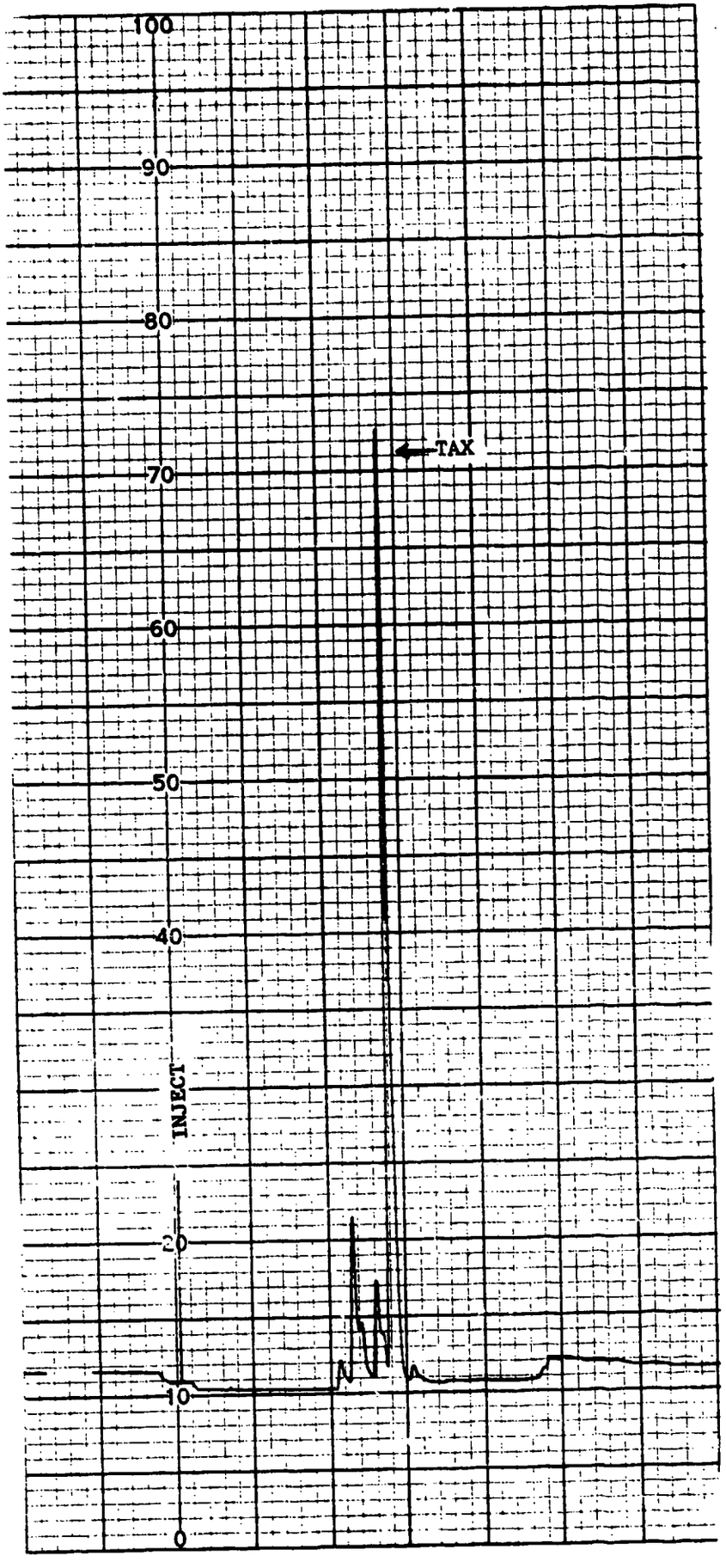


Figure 10 - 1009-67-7, 7X

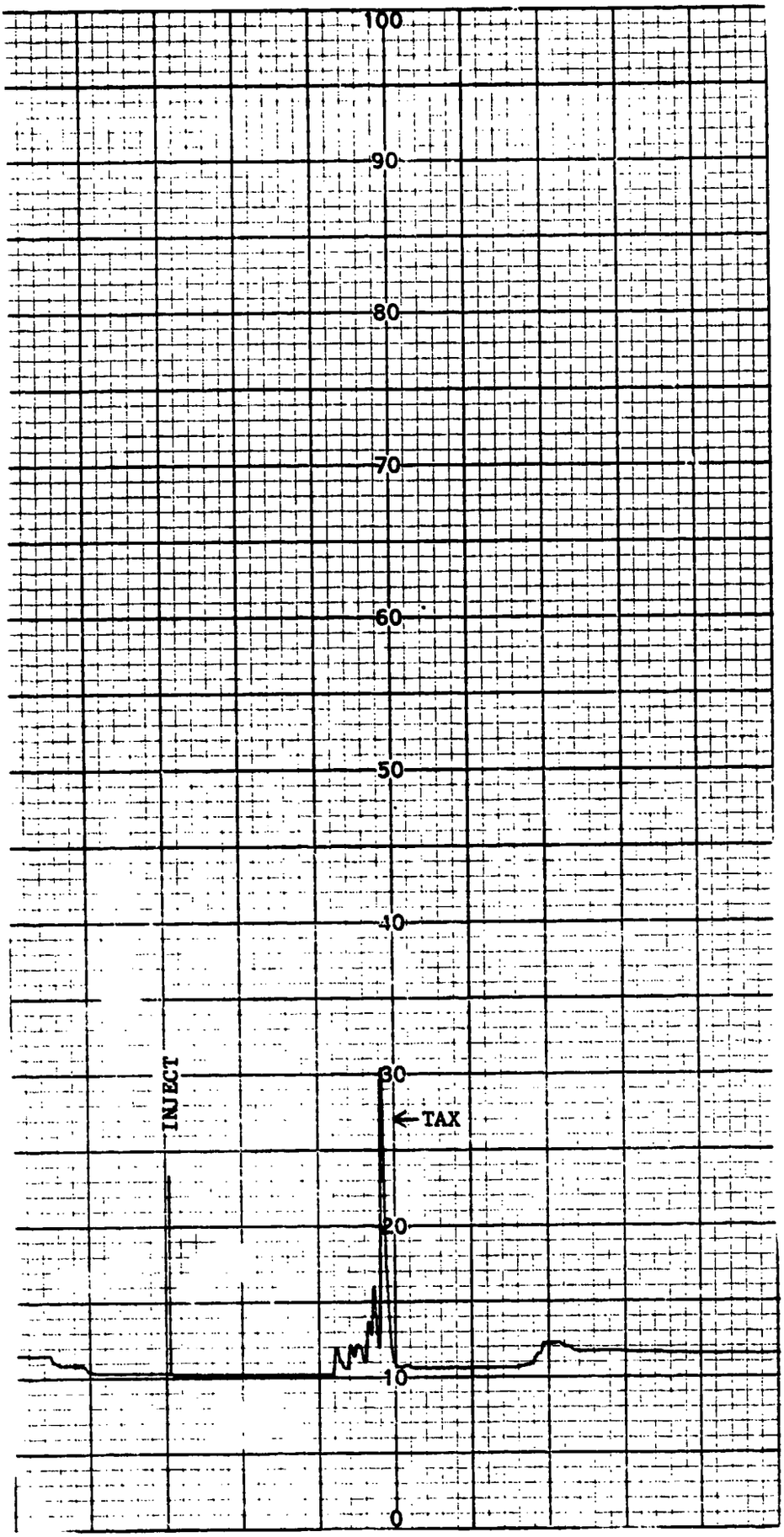


Figure 11 - 1009-67-7, Fraction 1

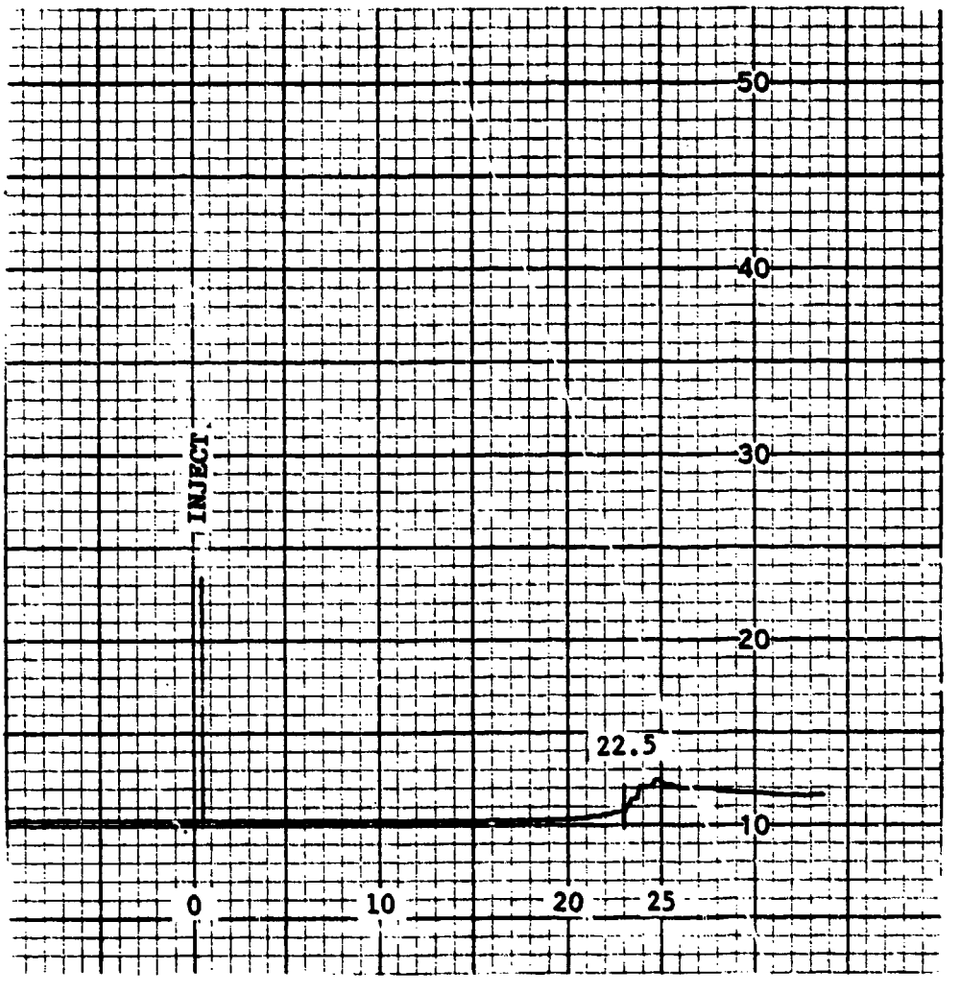


Figure 12 - Blank HPLC Run

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APPENDIX C

SAMPLE IDENTIFICATION STATUS ON HMX LINE UNKNOWNS

HMX Line Unknowns

No.	Holston AAP Sample Code	Identification Status		
		HPLC	Infrared Spectrum	Mass Spectrum
1	1009-73-2 fibrous residue (tissue filter)	a, b	c	a, b
2	1009-73-2 original IR5029 cyclohexanone dimer	a, b	No NNO_2 bonds	a No NNO_2 bonds
3	1009-73-4 IR5024/5 RDX	RDX	RDX	RDX
4	1009-73-4 acetone solubles	RDX is major component	c	Mixture containing RDX
5	1009-74-4 acetone insolubles	RDX	RDX	RDX
6	1009-73-5 acetone insoluble HMX, IR5045/46	c	HMX	HMX
7	1009-73-5 acetone solubles	HMX	HMX	HMX
8	1009-73-5 fibrous residue (tissue filter)	a, b	c	a, b
9	1009-73-5 original IR5026 HMX	HMX	HMX	c
10	1009-73-6 acetone solubles no IR scan	a, b	c	a
11	1009-73-6 acetone insolubles	c	c	SEX

HMX Line Unknowns (cont.)

No.	Holston AAP Sample Code	Identification Status HPLC	Infrared Spectrum	Mass Spectrum
12	1009-73-6 original IR5027, 5028 SEX	c	SEX	c
13	1009-73-6 1st LC fraction	c	SEX	c
14	1009-73-6 2nd LC fraction IR5053, 5044	a, Mixture	Mixture	a

a/ Studied but not identified.
b/ Too impure for identification.
c/ Not studied.

HMX Line Unknowns

No.	Holston AAP Sample Code	Identification Status HPLC	Infrared Spectrum	Mass Spectrum
1	1009-73-2 fibrous residue (tissue filter)	a, c	b	a, c
2	1009-73-2 original IR5029 cyclohexanone dimer	a, c	No NNO_2 bonds	a No NNO_2 bonds
3	1009-73-4 IR5024/5 RDX	RDX	RDX	RDX
4	1009-73-4 acetone solubles	RDX is major component	b	Mixture containing RDX
5	1009-74-4 acetone insolubles	RDX	RDX	RDX
6	1009-73-5 acetone insoluble HMX, IR5045/46	b	HMX	HMX
7	1009-73-5 acetone solubles	HMX	HMX	HMX
8	1009-73-5 fibrous residue (tissue filter)	a, c	b	a, c
9	1009-73-5 original IR5026 HMX	HMX	HMX	b
10	1009-73-6 acetone solubles no IR scan	a, c	b	a
11	1009-73-6 acetone insolubles	b	b	SEX

HMX Line Unknowns (cont.)

No.	Holston AAP Sample Code	Identification Status: HPLC	Infrared Spectrum	Mass Spectrum
12	1009-73-6 1st LC fraction	b	SEX	b
13	1009-73-6 original IR5027, 5028 SEX	b	SEX	b
14	1009-73-6 2nd LC fraction IR5053, 5044	a, Mixture	Mixture	a

a/ Studied but not identified.
b/ Not studied.
c/ Too impure for identification.

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APPENDIX D

DISCUSSION OF HMX LINE SAMPLES
WHICH WERE PARTIALLY CHARACTERIZED

Samples which could not be identified by study of their infrared spectrum or mass spectrum were examined by HPLC for purity. The HPLC conditions are the same as given in Appendix B.

Sample 1009-73-2, IR5029

HPLC (Figure 13) indicates numerous components. The IR spectrum was similar to that of cyclohexenylcyclohexanone with no indication of nitramines. A mass spectrum indicated strong ions at m/e 206 (100%) and 208 (88%). This sample will be reexamined by GC-MS using a capillary column.

Sample 1009-73-6, Acetone Solubles

HPLC (Figure 14) indicates several components in significant concentration. One component had the retention time of SEX. Mass spectral data were not definitive except to indicate the presence of nitramines.

Sample 1009-73-6, 2nd LC Fraction

Although several components were observed by HPLC (Figure 16) one peak was much larger than the others. Infrared spectrum shows absorptions similar to those in cyclohexenylcyclohexanone plus 3050 and 3020 cm^{-1}, indicating nitramines. Mass spectral examination has not given useful results.

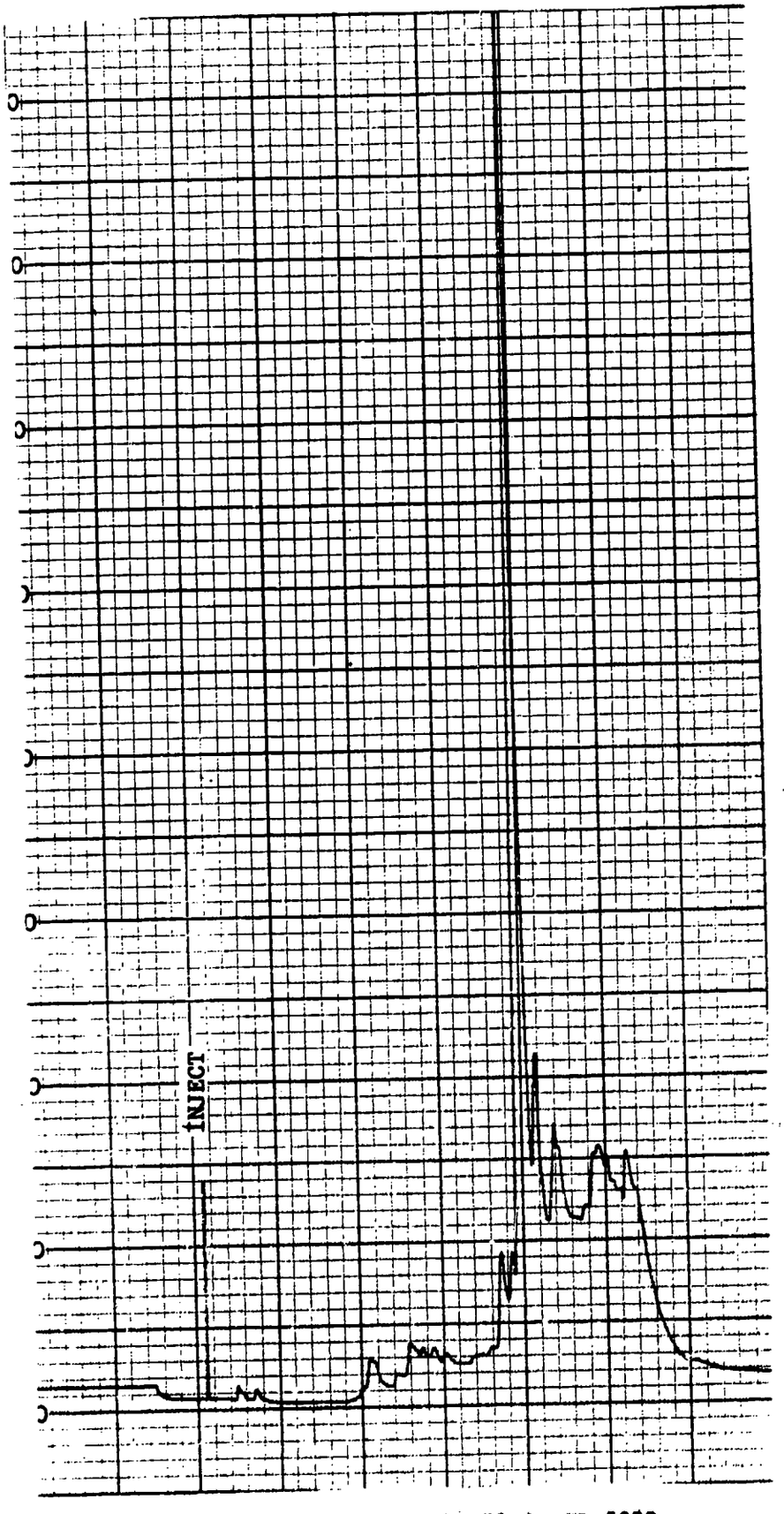

Figure 13 - 1009-73-2, IR 5029

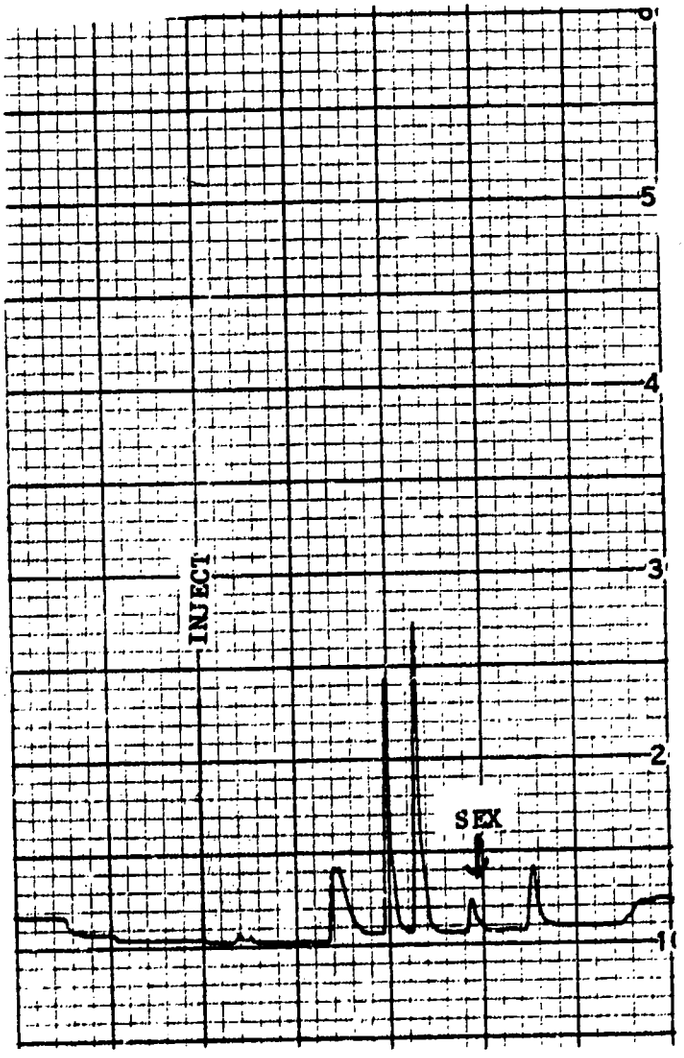

Figure 14 - 1009-73-6, Acetone Solubles

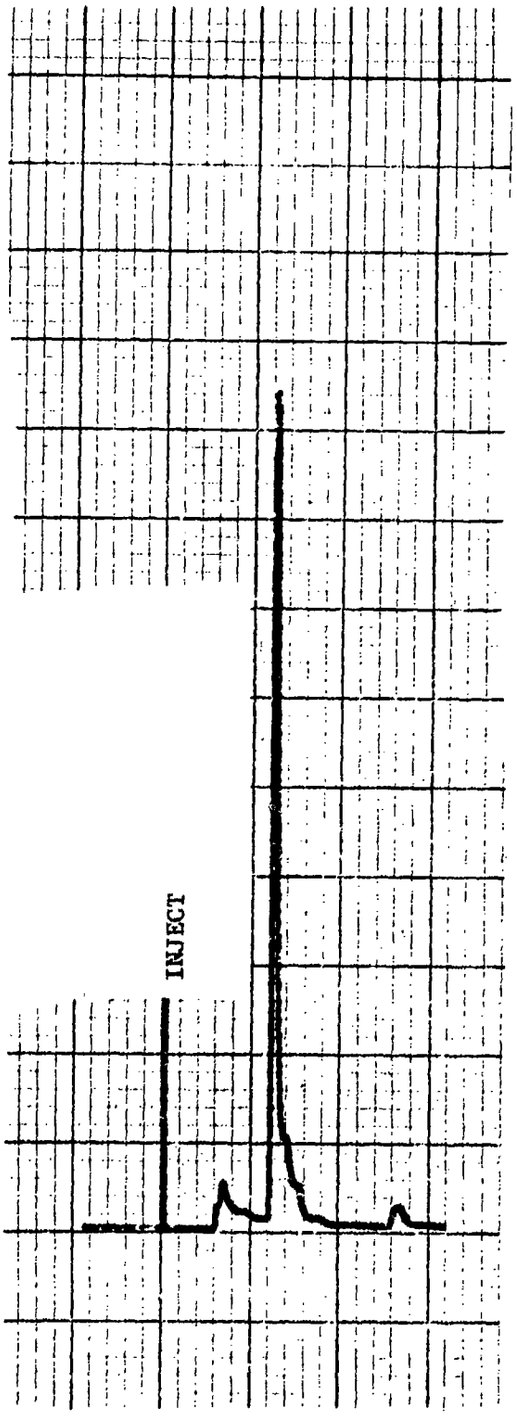

Figure 15 - 1009-73-6, 2nd LC Fraction (Slightly Different IC Conditions)

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APPENDIX E

REFERENCE SAMPLES

Reference Samples

No.	Sample Code	Mass Spectrum Observed	Satisfactory Spectrum	Spectrum Interpreted
1	H-6	Yes	Yes	Yes
2	HAMN	Yes	Yes	Yes
3	"106"	Yes	No	-
4	TTP	Yes	Yes	Yes
5	AcAn	Yes	Yes	Yes
6	PHX-An	Yes	Yes	Yes
7	H-2	Yes	Yes	Yes
8	HADN	Yes	Yes	Yes
9	RDX	Yes	Yes	Yes
10	HMX	Yes	Yes	Yes
11	DPT	Yes	Yes	Yes
12	H-16	Yes	Yes	Yes
13	Compound C	Yes	No	-
14	BSX	Yes	No	-
15	PHX	Yes	No	-
16	SEX	Yes	Yes	Yes

APPENDIX C

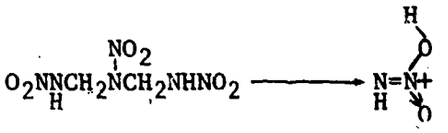
FIELD IONIZATION MASS SPECTRA OF NITRAMINES

APPENDIX C[a/]

I. Field Ionization Mass Spectra of Reference Samples

Each fragment molecule carries a positive cahrge; however, this charge is sometimes not formally indicated due to undertainty as to which atom carries the charge.

A. TTP (Sample 036)



MW 210
m/e 210 (0% intensity)

CH_2NNO_2 + CH_2NNO_2

75 (34%) 74 (100%)

NO_2

46 (83%)

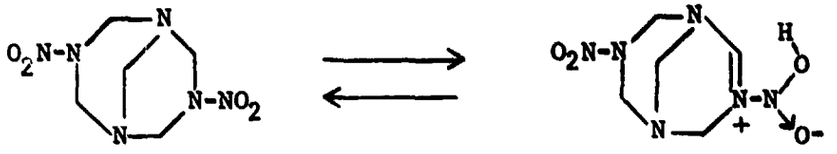
$C_1H_2N_3O_4$

120 (9%)

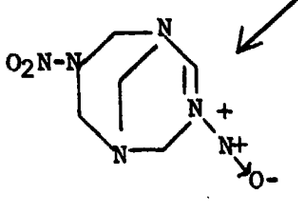
---

a/ In some cases alternate fragmentation patterns can account for the observed ions.

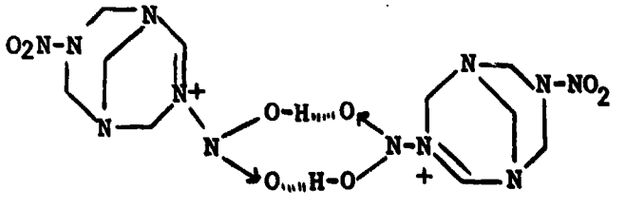
B. DPT (Sample No. 002)



DPT
MW 218
m/e 218 (100% intensity)
218 + 1 (17%)

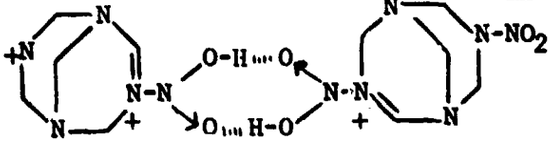


201 (1.4%)



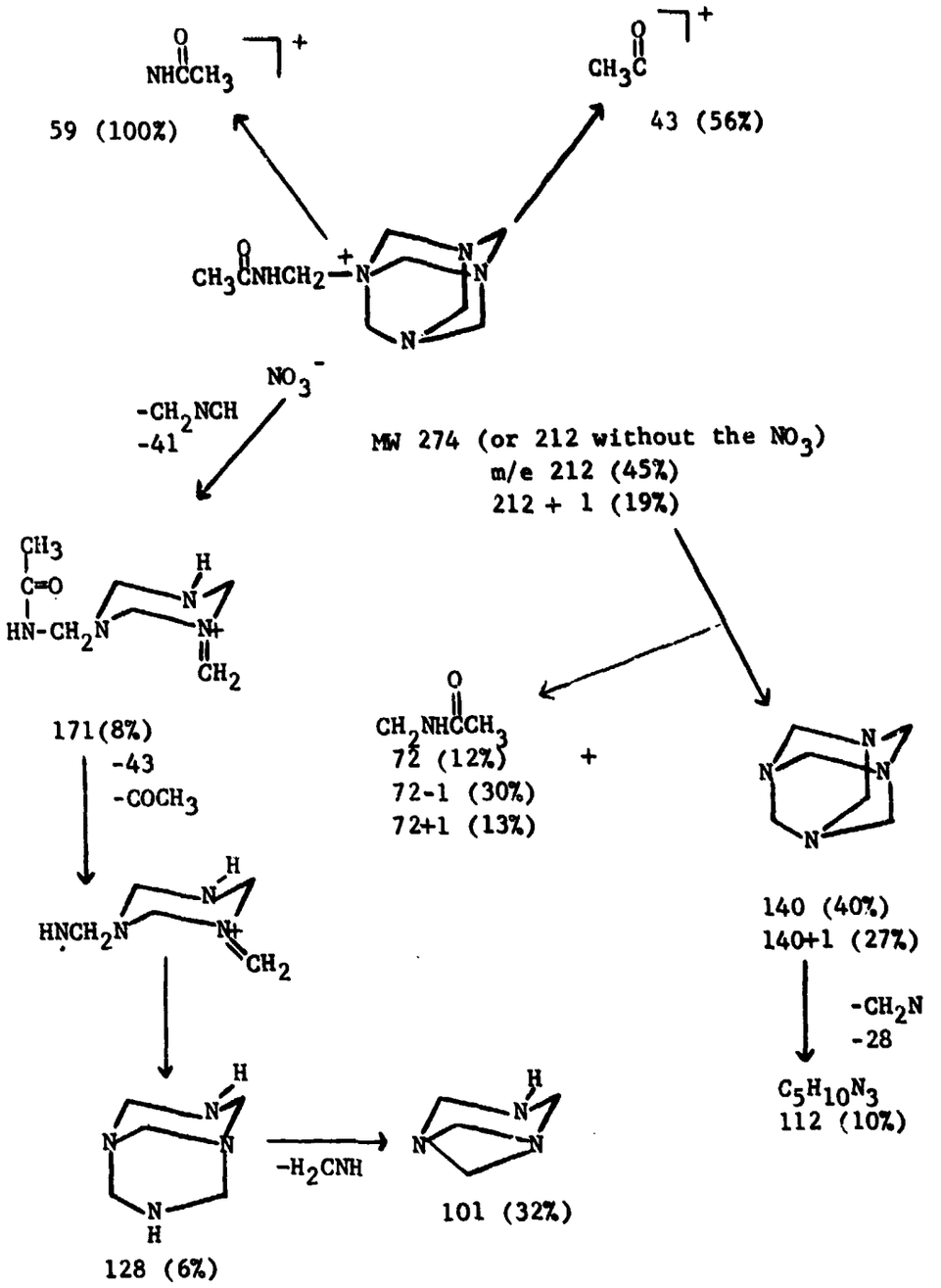
2M = 436 (10%)
2M + 1 = 437 (7%)

$-NO_2$

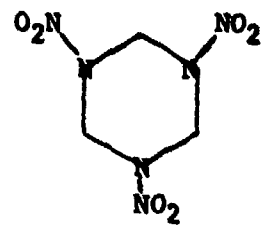


390 (2.5%)

C. H-2 (Sample No. 022)

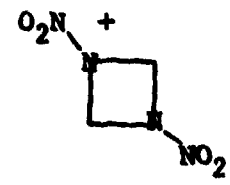


D. RDX (Sample No. 037)



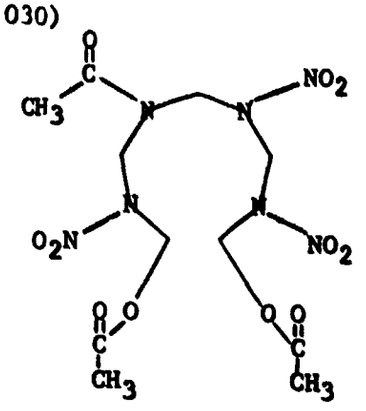
MW 222

2M + 1 445 (6%)
M + 1 223 (4%) $\xrightarrow{-CH_2NNO_2,\ -74}$



149 (1%)

E. H-16 (Sample No. 030)

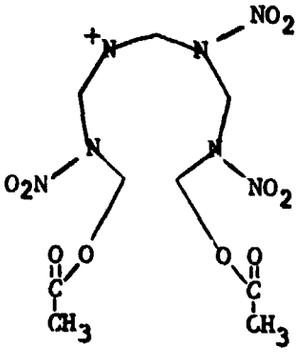


MW 425
m/e 425 (intensity 0%)

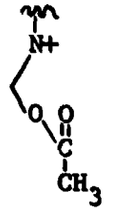
-43
$-COCH_3$

-46
$-NO_2$

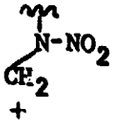
-59
$-O-COCH_3$



382 (8%)

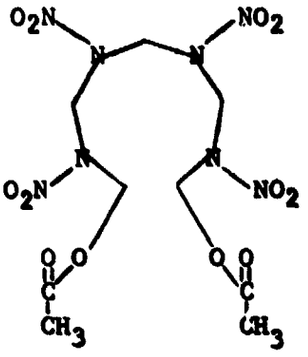


379 (100%)



366 (5%)

F. AcAn (Sample No. 004)

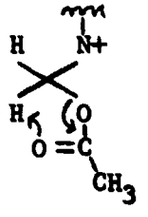


MW 428

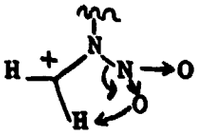
m/e 428 (0% intensity)

$-NO_2$ -46

$-O-\overset{O}{\overset{\|}{C}}-CH_3$ -59



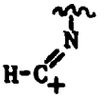
m/e 382 (51%)



m/e 369 (100%)

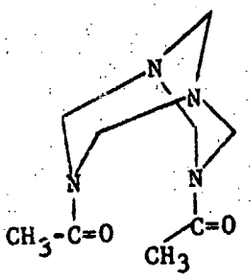
$-HOCOCH_3$ -60

$-HNO_2$ -47



322 (29%)

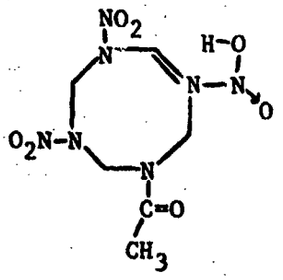
G. H-6 (Sample No. 024)



MW 212

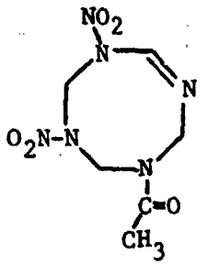
m/e 212 (intensity 100%)
m/e 213 (15%)

H. SEX (Sample No. 010)



M = 293 (0% intensity)
M + 1 = 294 (17%)
2M + 1 = 587 (10%)

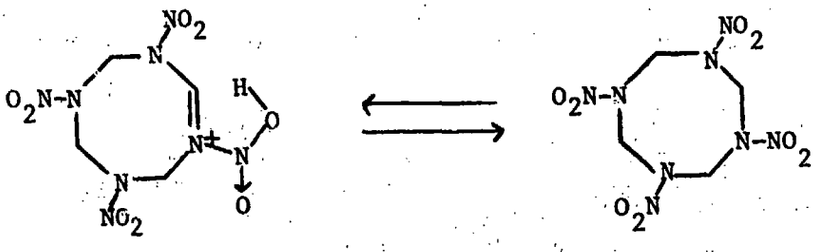
$-HNO_2$



247 (49%)

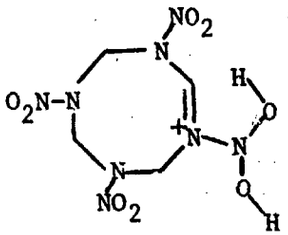
$\overset{O}{\overset{\|}{C}}CH_3$ (100%)

I. HMX (Sample No. 001)



MW 296.2
m/e (% intensity)
M = 296 (0%)

+H

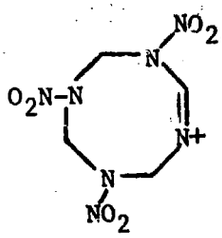


M + 1 = 297 (5%)

$-NO_2$

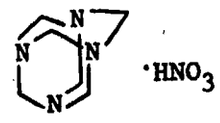
NO_2
46 (100%)

HONOH
48 (5%)



249 (5%)

J. HAMN (Sample No. 038)



M = 140 (100%) organic moiety
M + 1 = 141 (6.5%)

HNO_3
63 (35%)

NO_2
46 (4%)

APPENDIX D

IDENTIFICATION OF CYCLOHEXANONE COMPONENTS

January 5, 1978

Major Illar Muul
Attn: SGRD-UBG
Environmental Protection Department
U.S. Army Medical Bioengineering
Research and Development Laboratory
Fort Detrick
Frederick, Maryland 21701

Subject: Subtask 9, Identification of Waste Products from RDX and HMX Manufacture, Monthly Report No. 16.

Name of Contractor: Midwest Research Institute
425 Volker Boulevard
Kansas City, Missouri 64110

Contract Number: DAMD-17-74-C-4073 Mod. 708

Program Director: Dr. Cheng-Chun Lee

Phone Number: 816-753-7600

Date of Report: January 5, 1978

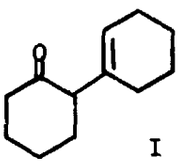
Period Covered: October 1 through December 30, 1977

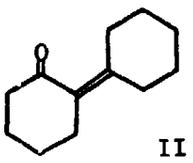
Gentlemen:

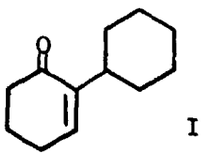
The recently received cyclohexanone wastewater sample and cyclohexanone distillate have been examined by capillary gas chromatography-mass spectroscopy (GC-MS). Several of the partially purified samples submitted earlier by Holston AAP were also examined. Each group of samples is discussed below, plus future work and costs to date.

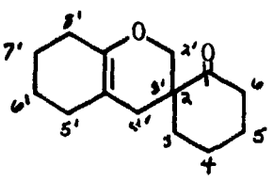
1. Cyclohexanone Wastes

Low resolution GC-MS data were obtained using a Finnigan 4000 coupled to a 30 meter capillary GC column coated with SE-30. High resolution data were obtained using a 3% OV-1 on Chromasorb W column coupled to a Varian MAT 311A. Two samples (Code No. DHC-2 and DHCYHEXDIS) were examined. By mass spectrum both samples contain 2-(1-cyclohexenyl)-cyclohexanone (I), 2-cyclohexylidenecyclohexanone (II), 2-cyclohexylcyclohex-2-enone (III, spiro[1-oxocyclohexane-2,3'-2',4',5',6',7',8'-hexahydrobenzo[(b)]pyran] (IV) or spiro[1-oxocyclohexane-2,2'-3',4',5',6',7',8'-hexahydrobenzo[(b)]pyran] (V).

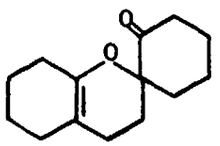

I

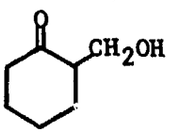

II


III



IV


V



VI

Sample C-2 also contains 2-hydroxymethylcyclohexanone (VI). Samples of I through VI are currently being synthesized for structural proof and for use in quantitating their concentration in the effluent. The origin of each sample and a detailed analysis of its GC-MS is given below.

A. Sample DHCYHEXDIS

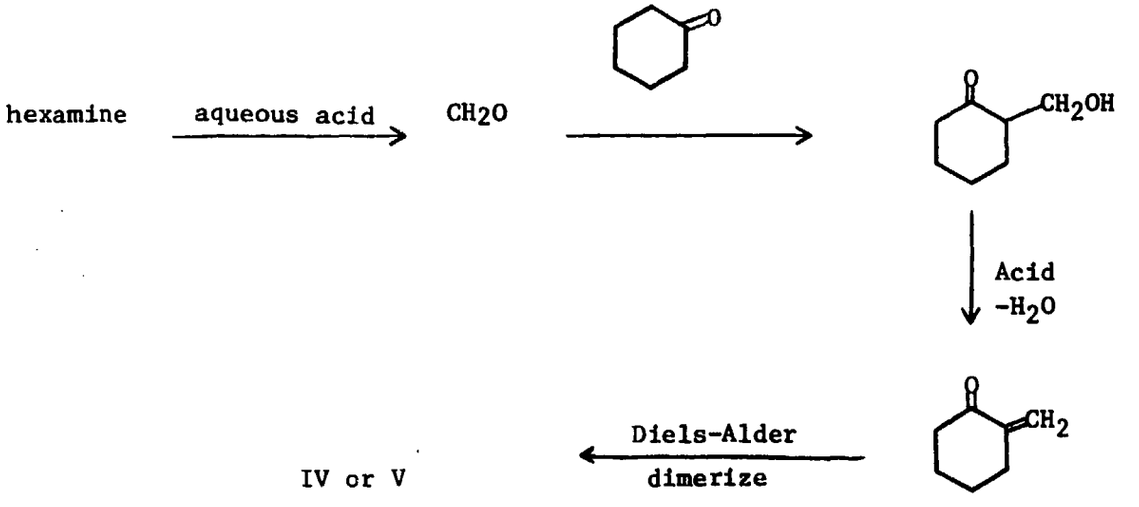
Cyclohexanone used for recrystallization is purified by azeotropic distillation with water. Sample DHCYHEXDIS is an aliquot of the cyclohexanone layer from this distillation. Figure 1 shows a reconstructed gas chromatogram (RGC) using the mass spectrometer as a detector. The y-axis is calibrated in arbitrary units so as to normalize the highest peak. The x-axis is calibrated in both time in minutes (0 to 28) and spectrum scan number (0 to 1400). GC column temperature was 100° for 5 minutes, followed by programming at 8°/minute to 200° and hold.

The mass spectrum of the peak at scan number 160 was identified as cyclohexanone based on comparison with the spectrum of authentic cyclohexanone (Figure 2).

Figure 3 shows a scan expansion in the scan region of 700 to 1400 with normalization on the most intense peak in that region. Baseline drift

is noted in the scan region 750 to 840. The baseline drop at scan 840 corresponds roughly to the end of the temperature program. Study of scan 800, a region free of peaks, indicates the presence of residual cyclohexanone. Thus peaks at scans 767-8, 785 and 836-7 were corrected for the presence of cyclohexanone by subtracting scan 800. Study of scans 767 to 768 (Figures 4 and 5), 785 (Figures 6 and 7), and 836 to 837 (Figures 8 and 9) indicated the presence of three components of mass 178 having a similar fragmentation pattern. Comparison of these fragmentation patterns with that a sample supplied by Holston AAP containing a mixture of I, II, and unknowns indicates the presence of I and II. Examination of these three peaks by high resolution indicates each have the same molecular weight, suggesting structures I, II, and III. It is not yet possible to assign structures to individual peaks.

Scan 920 (Figures 10 and 11) is consistent with the presence of IV or V. Examination of this peak by high resolution indicates the m/e 220 peak has an elemental composition consistent with IV or V. Observed mass minus calculated mass equals 0.5 millimass units. IV or V can arise by the following process:



The peak at scan 1084 was examined; however, the ion intensities were too close to that of background to produce unambiguous results.

B. Sample DHC2

A sample of the Holston AAP Building H-2 wastewater was extracted with chloroform for 24 hr using a continual extractor. Cooling of the chloroform to room temperature produced a white amorphorus precipitate which was removed by filtration and retained for later examination. The chloroform solubles were examined by GC-MS as described under A above.

Figure 12 shows a reconstructed gas chromatograph trace for sample DHC2. The first major peak near scan 100 is due to the chloroform solvent, and the next major peak near scan 160 is due to cyclohexanone. Scan 392 is consistent with the presence of 2-hydroxymethylcyclohexanone (VI) (Figures 13 and 14). Comparison of scan 392 to the INCOS mass spectral data file gave a computer fit for 2-hydroxymethylcyclohexanone.

The mass spectrum of a synthetic reference sample of 2-hydroxymethylcyclohexanone[1/] was similar to that of scan 392 and the synthetic sample had a similar GC retention time.

Figure 15 shows sample DHC2 with scale expansion for scans 600 to 1200. The peaks at 767, 785, and 839 are consistent with structures I, II, and III (elution order unknown). The peak at 918 is consistent with IV or V.

The identity of the remaining peaks is being studied.

II. Partially Purified Samples Submitted by Holston AAP

Six samples were studied by capillary GC-MS as described in I. Most of the data is complex and requires further analysis. Figure 16, 17, and 18 indicate the complexity of some of the samples.

III. Future Work

As required the data available will be studied further. When a tentative identification has been made, high resolution data may be obtained and a reference sample prepared. When reference samples are prepared, the

1/ Elaginn, N. V., N. S. Martinkova, and B. A. Kazanskii (State University, Moscow) Zhur. Obshchei Khim., 29, 4011 (1959).

goal will be to positively identify the unknown and provide a small reference sample for use by Holston AAP in routine monitoring.

IV. Costs to Date

As of December 31, $10,390 remained. This includes funds for one trip to Holston AAP.

Sincerely,

MIDWEST RESEARCH INSTITUTE

Dan Helton

Danny O. Helton
Senior Chemist

Approved:

W. B. House, Director
Biological Sciences Division

(15 copies of report submitted)

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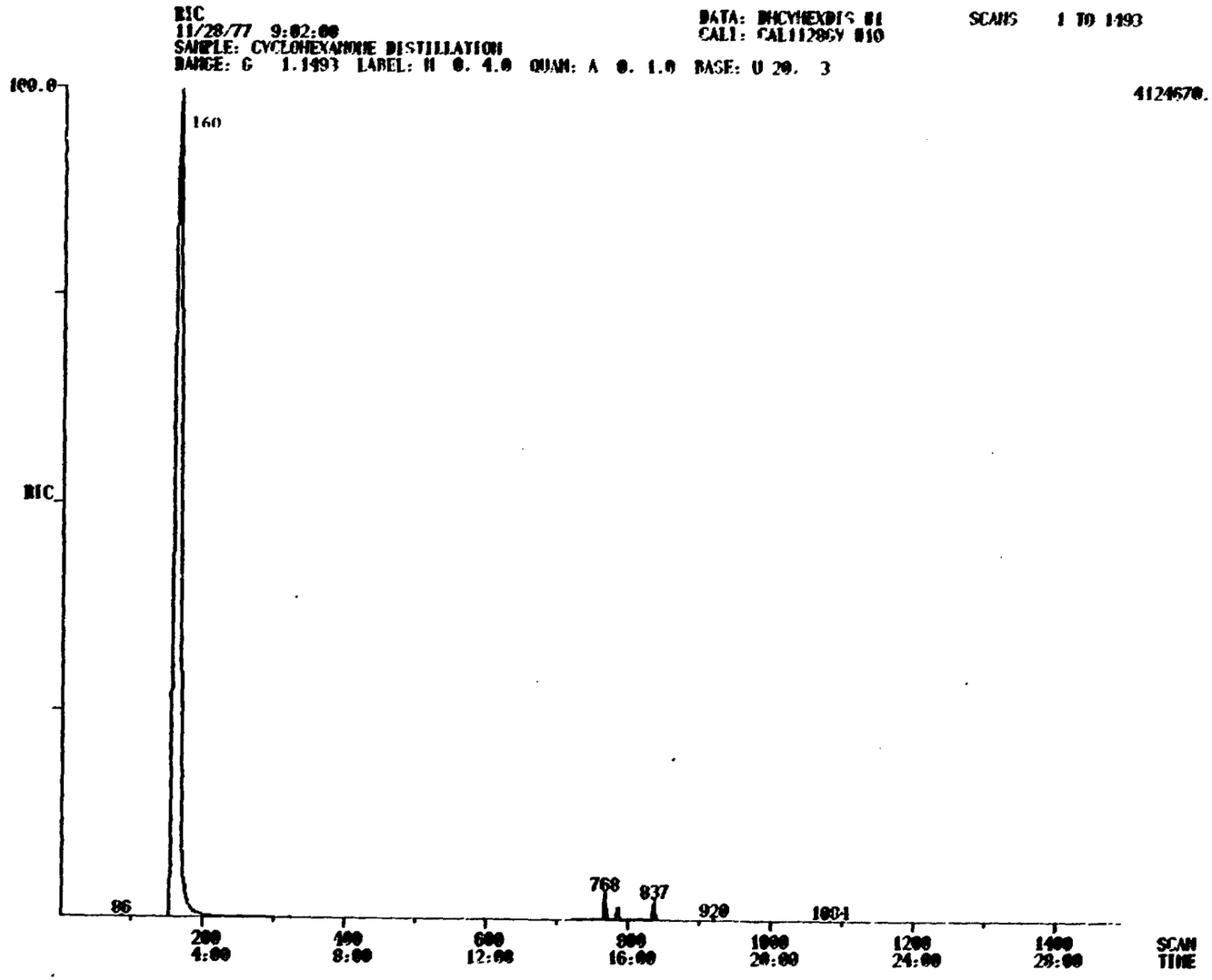


Figure 1 - Sample DHCYHEXDIS

LOW RESOLUTION MASSES SAMPLE RUN: DHREF1027 SCANS 229 TO 231
DATE: 10/27/77 TIME: 1042 CALIB. RUN: CAL1027 -SCANS 220 TO 225
SAMPLE: C6-H10-01 BASE M/E: 55 TOTAL IONIZATION: 196825.
LIST THRESHOLD = 1.00 % RELATIVE ABUNDANCE

PEAK NO.	NOMINAL MASS	% RA	% TI	INT
1	26?	9.41	1.82	3572.
2	27?	46.80	9.06	17759.
3	28	19.56	3.79	7423.
4	29	13.11	2.54	4975.
12	37	1.80	0.35	685.
13	38	4.78	0.93	1816.
14	39	38.49	7.45	14607.
15	40	9.02	1.75	3424.
16	41	46.80	9.06	17759.
17	42	95.95	18.58	36415.
18	43	13.03	2.52	4943.
19	44	1.15	0.22	438.
24	50	2.08	0.40	789.
25	51	2.50	0.48	948.
27	53	3.80	0.74	1442.
28	54	7.35	1.42	2788.
29	55	100.00	19.36	37951.
30	56	11.17	2.16	4239.
31	57	1.33	0.26	505.
40	68	1.03	0.20	391.
41	69	22.60	4.37	8575.
42	70	16.72	3.24	6343.
43	71	1.32	0.26	502.
48	80	2.98	0.58	1130.
50	83	5.98	1.16	2268.
53	97	1.58	0.31	600.
54	98	25.97	5.03	9855.
55	99	1.75	0.34	666.

Total Intensity Observed

Percentage of Total Intensity

Normalized Percent Ionization

Figure 2 - Cyclohexanone

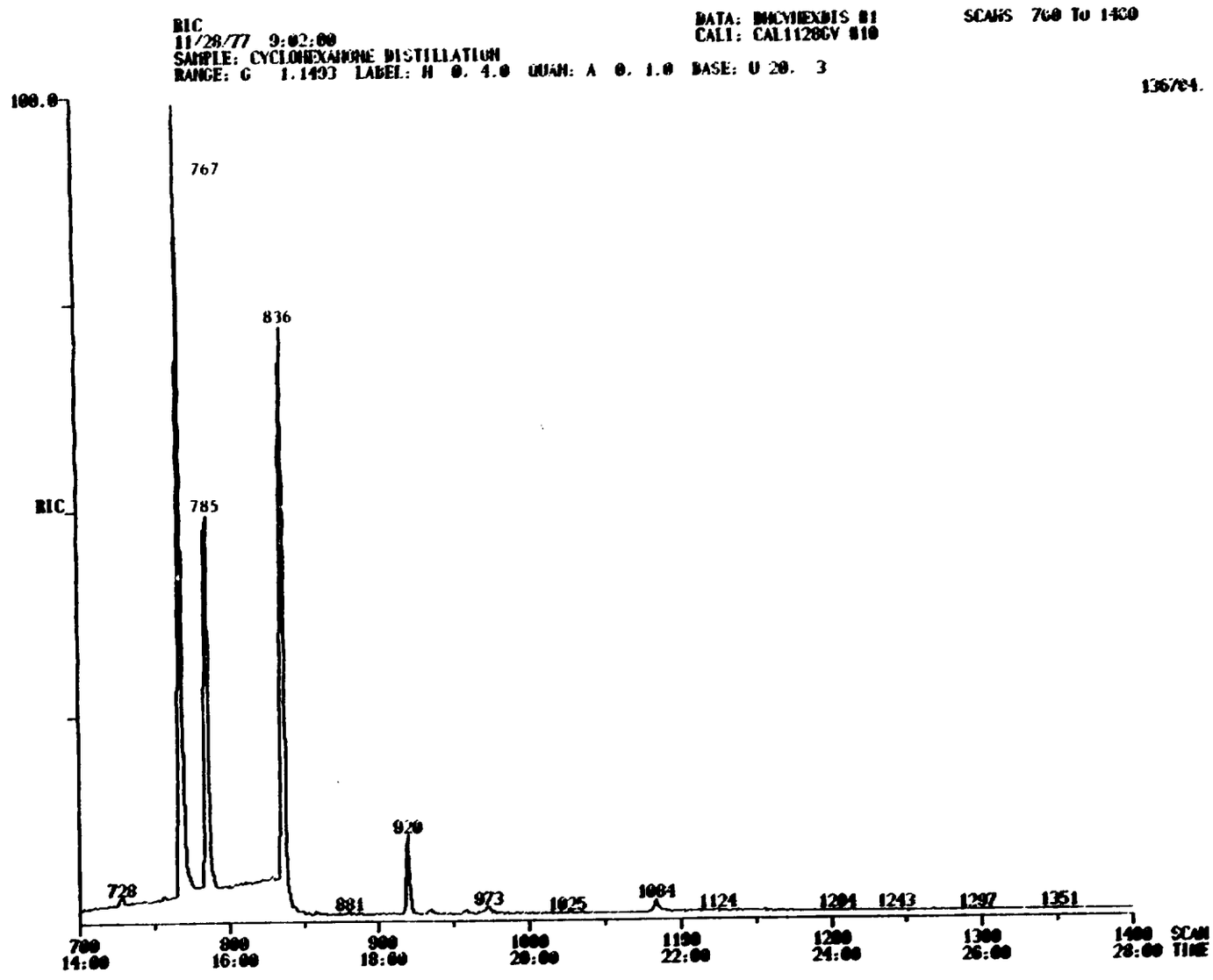


Figure 3 - Sample DHCYHEXDIS

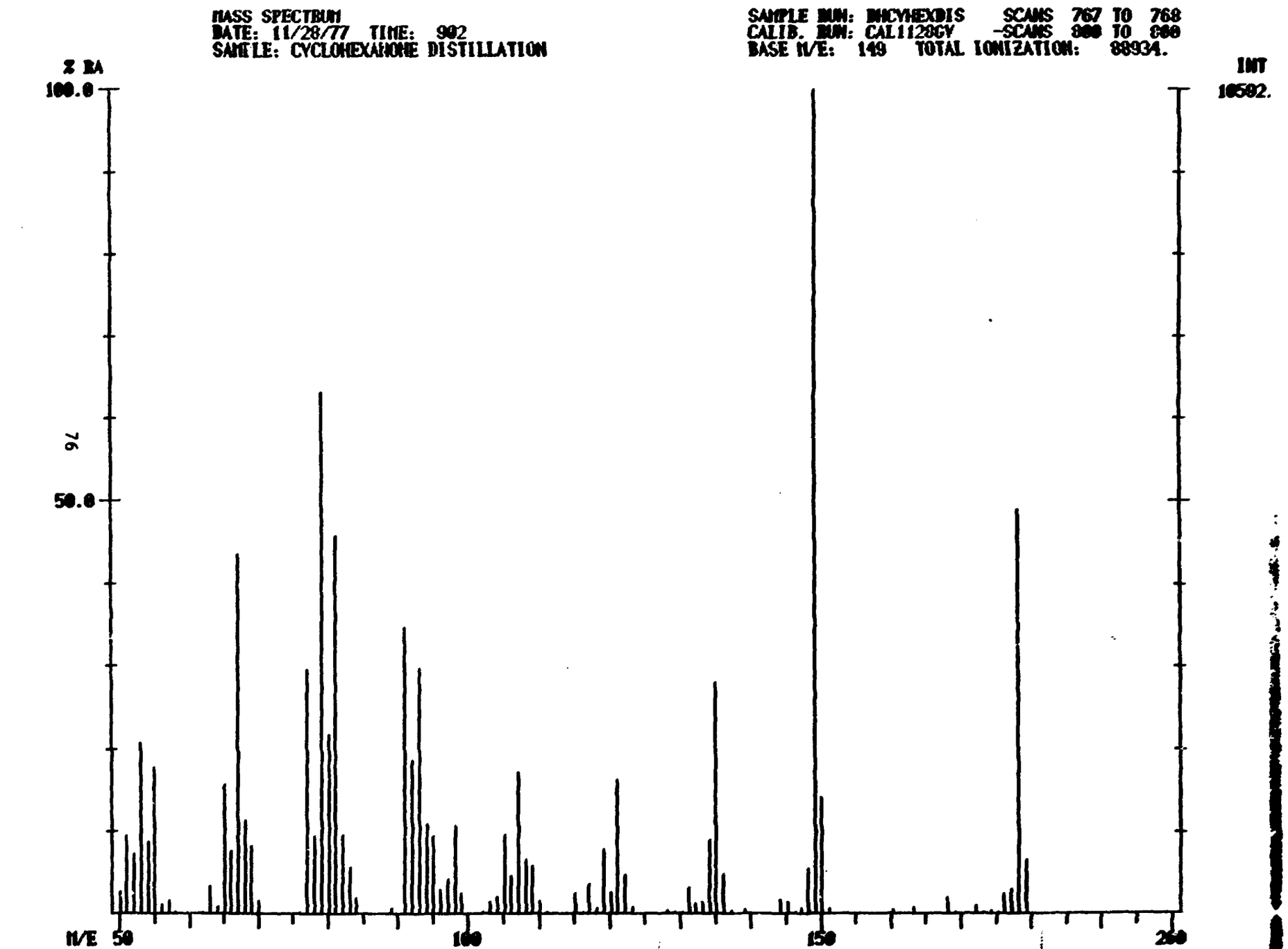


Figure 4 - Scan 767, Bar Graph

LOW RESOLUTION MASSES SAMPLE RUN: DHCYHEXDIS SCANS 767 TO 768
DATE: 11/28/77 TIME: 902 CALIB. RUN: CAL1128GV -SCANS 800 TO 800
SAMPLE: CYCLOHEXANONE DISTILBASE M/E: 149 TOTAL IONIZATION: 88934.
LIST THRESHOLD = 1.00 % RELATIVE ABUNDANCE

PEAK NO.	NOMINAL MASS	% RA	% TI	INT
1	42?	33.12	3.94	3508.
2	43?	5.22	0.62	553.
4	50	2.56	0.30	271.
5	51	9.47	1.13	1003.
6	52	7.20	0.86	763.
7	53	20.69	2.46	2192.
8	54	8.66	1.03	917.
9	55	17.73	2.11	1878.
10	56	1.09	0.13	115.
11	57	1.52	0.18	161.
14	63	3.37	0.40	357.
16	65	15.67	1.87	1660.
17	66	7.71	0.92	817.
18	67	43.50	5.18	4607.
19	68	11.39	1.36	1206.
20	69	8.22	0.98	871.
21	70	1.60	0.19	169.
23	77	29.65	3.53	3140.
24	78	9.43	1.12	999.
25	79	63.14	7.52	6687.
26	80	21.71	2.59	2300.
27	81	45.77	5.45	4847.
28	82	9.58	1.14	1015.
29	83	5.70	0.68	604.
30	84	1.81	0.22	192.
32	91	34.63	4.12	3668.
33	92	18.60	2.22	1970.
34	93	29.76	3.54	3152.
35	94	10.86	1.29	1150.
36	95	9.48	1.13	1004.
37	96	2.82	0.34	299.
38	97	4.19	0.50	444.
39	98	10.74	1.28	1138.
40	99	2.49	0.30	264.
42	103	1.44	0.17	153.
43	104	1.94	0.23	206.
44	105	9.60	1.14	1017.
45	106	4.68	0.56	496.
46	107	17.20	2.05	1822.
47	108	6.62	0.79	701.
48	109	5.86	0.70	621.
49	110	1.51	0.18	160.
51	115	2.52	0.30	267.
53	117	3.57	0.43	378.
55	119	7.82	0.93	828.
56	120	2.61	0.31	276.
57	121	16.26	1.94	1722.
58	122	4.80	0.57	508.
63	131	3.20	0.38	339.
64	[illegible]	1.20	0.14	127.
65	133	1.43	0.17	151.
66	134	8.98	1.07	951.
67	135	27.91	3.32	2956.

Figure 5 - Scan 767-8, Peak List

PEAK NO.	NOMINAL MASS	% RA	% TI	INT
68	136	4.96	0.59	525.
71	144	1.77	0.21	187.
72	145	1.39	0.17	147.
75	148	5.54	0.66	587.
76	149	100.00	11.91	10591.
77	150	14.22	1.69	1506.
81	168	1.96	0.23	208.
83	172	0.99	0.12	105.
87	176	2.42	0.29	256.
88	177	2.98	0.36	316.
89	178	48.87	5.82	5175.
90	179	6.60	0.79	699.

Figure 5 (concluded)

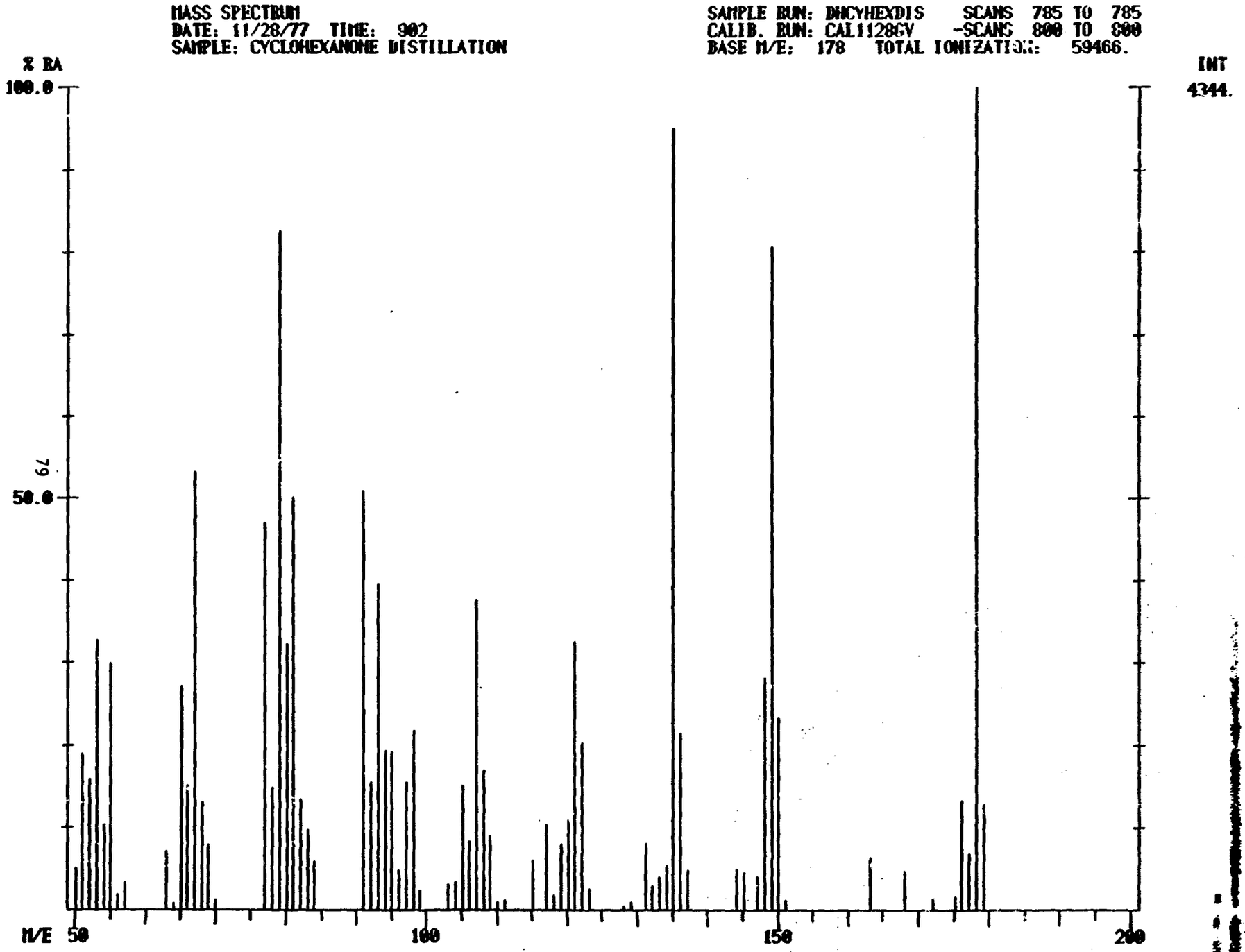


Figure 6 - Scan 785, Bar Graph

LOW RESOLUTION MASSES SAMPLE RUN: DHCYHEXDIS SCANS 785 TO 785
DATE: 11/28/77 TIME: 902 CALIB. RUN: CAL1128GV -SCANS 800 TO 800
SAMPLE: CYCLOHEXANONE DISTILBASE M/E: 178 TOTAL IONIZATION: 59466.
LIST THRESHOLD = 1.00 % RELATIVE ABUNDANCE

PEAK NO.	NOMINAL MASS	% RA	% TI	INT
1	42?	41.94	3.06	1822.
2	43?	12.94	0.88	523.
4	50	5.11	0.37	222.
5	51	18.85	1.38	819.
6	52	15.77	1.15	685.
7	53	32.78	2.39	1424.
8	54	10.36	0.76	450.
9	55	29.88	2.18	1298.
10	56	1.84	0.13	80.
11	57	3.34	0.24	145.
12	63	7.02	0.51	305.
14	65	27.07	1.98	1176.
15	66	15.03	1.10	653.
16	67	53.22	3.89	2312.
17	68	13.10	0.96	569.
18	69	7.90	0.58	343.
19	70	1.29	0.09	56.
20	77	46.92	3.43	2038.
21	78	14.80	1.08	643.
22	79	82.60	6.03	3588.
23	80	32.27	2.36	1402.
24	81	50.00	3.65	2172.
25	82	13.40	0.98	582.
26	83	9.69	0.71	421.
27	84	5.87	0.43	255.
28	91	50.83	3.71	2208.
29	92	15.47	1.13	672.
30	93	39.59	2.89	1720.
31	94	19.36	1.41	841.
32	95	19.15	1.40	832.
33	96	4.79	0.35	208.
34	97	15.52	1.13	674.
35	98	21.66	1.58	941.
36	99	2.42	0.18	105.
37	103	3.25	0.24	141.
38	104	3.50	0.26	152.
39	105	14.99	1.09	651.
40	106	8.31	0.61	361.
41	107	37.62	2.75	1634.
42	108	17.01	1.24	739.
43	109	9.00	0.66	391.
45	111	1.29	0.09	56.
46	115	6.01	0.44	261.
47	117	10.36	0.76	450.
48	118	1.89	0.14	82.
49	119	8.03	0.59	349.
50	120	10.87	0.79	472.
51	121	32.60	2.38	1416.
52	122	20.30	1.48	882.
53	123	2.65	0.19	115.
55	129	1.01	0.07	44.
56	131	8.13	0.59	353.
57	132	3.02	0.22	131.

Figure 7 - Scan 785, Peak List

PEAK NO.	NOMINAL MASS	% RA	% TI	INT
58	133	4.14	0.30	180.
59	134	5.48	0.40	238.
60	135	95.03	6.94	4127.
61	136	21.48	1.57	933.
62	137	4.79	0.35	208.
63	144	5.00	0.36	217.
64	145	4.47	0.33	194.
65	147	4.03	0.29	175.
66	148	28.08	2.05	1220.
67	149	80.57	5.89	3500.
68	150	23.32	1.70	1013.
69	151	1.20	0.09	52.
70	163	6.45	0.47	280.
71	168	4.70	0.34	204.
72	172	1.43	0.10	62.
73	175	1.70	0.12	74.
74	176	13.31	0.97	578.
75	177	6.93	0.51	301.
76	178	100.00	7.30	4343.
77	179	12.82	0.94	557.

Figure 7 (concluded)

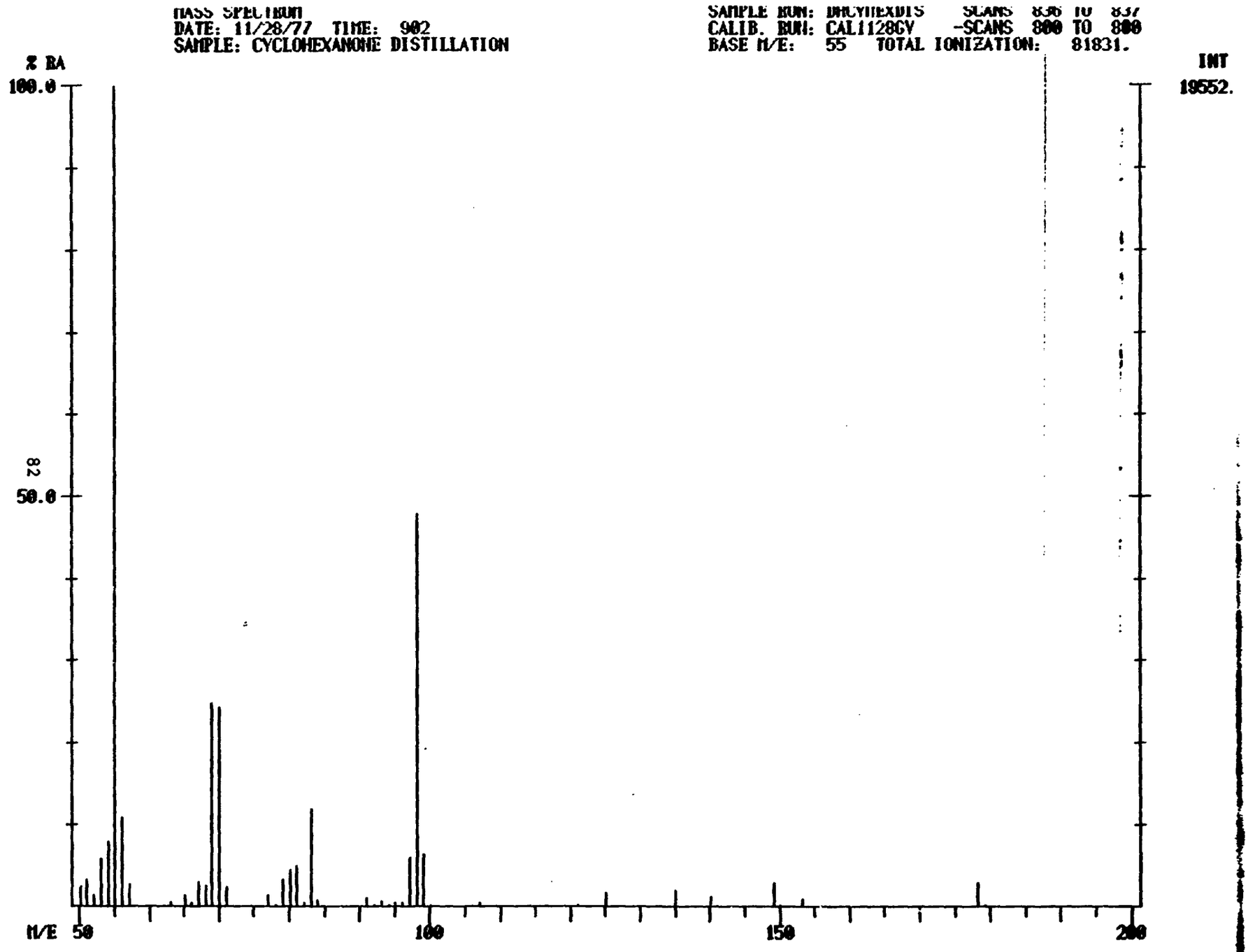


Figure 8 - Scan 836-7, Bar Graph

LOW RESOLUTION MASSES SAMPLE RUN: DHCYHEXDIS SCANS 836 TO 837
DATE: 11/28/77 TIME: 902 CALIB. RUN: CAL1128GV -SCANS 000 TO 000
SAMPLE: CYCLOHEXANONE DISTILBASE M/E: 42 TOTAL IONIZATION: 81031.
LIST THRESHOLD = 1.00 % RELATIVE ABUNDANCE

PEAK NO.	NOMINAL MASS	% RA	% TI	INT
1	42?	100.00	25.11	20543.
2	43?	13.75	3.45	2824.
5	50	2.27	0.57	466.
6	51	3.18	0.80	654.
7	52	1.31	0.33	269.
8	53	5.54	1.39	1138.
9	54	7.54	1.89	1550.
10	55	95.17	23.89	19551.
11	56	10.34	2.60	2124.
12	57	2.56	0.64	525.
14	65	1.33	0.33	273.
16	67	2.91	0.73	597.
17	68	2.50	0.63	513.
18	69	23.44	5.89	4915.
19	70	23.05	5.79	4735.
20	71	2.32	0.58	476.
21	77	1.39	0.35	286.
23	79	3.21	0.81	660.
24	80	4.33	1.09	889.
25	81	4.77	1.20	980.
27	83	11.31	2.84	2324.
29	91	1.12	0.28	231.
35	97	5.71	1.43	1174.
36	98	45.56	11.44	9359.
37	99	6.09	1.53	1252.
41	125	1.56	0.39	321.
42	135	1.87	0.47	384.
43	140	1.25	0.31	256.
44	149	2.69	0.67	552.
47	178	2.77	0.70	570.

Figure 9 - Scan 836-7, Peak List

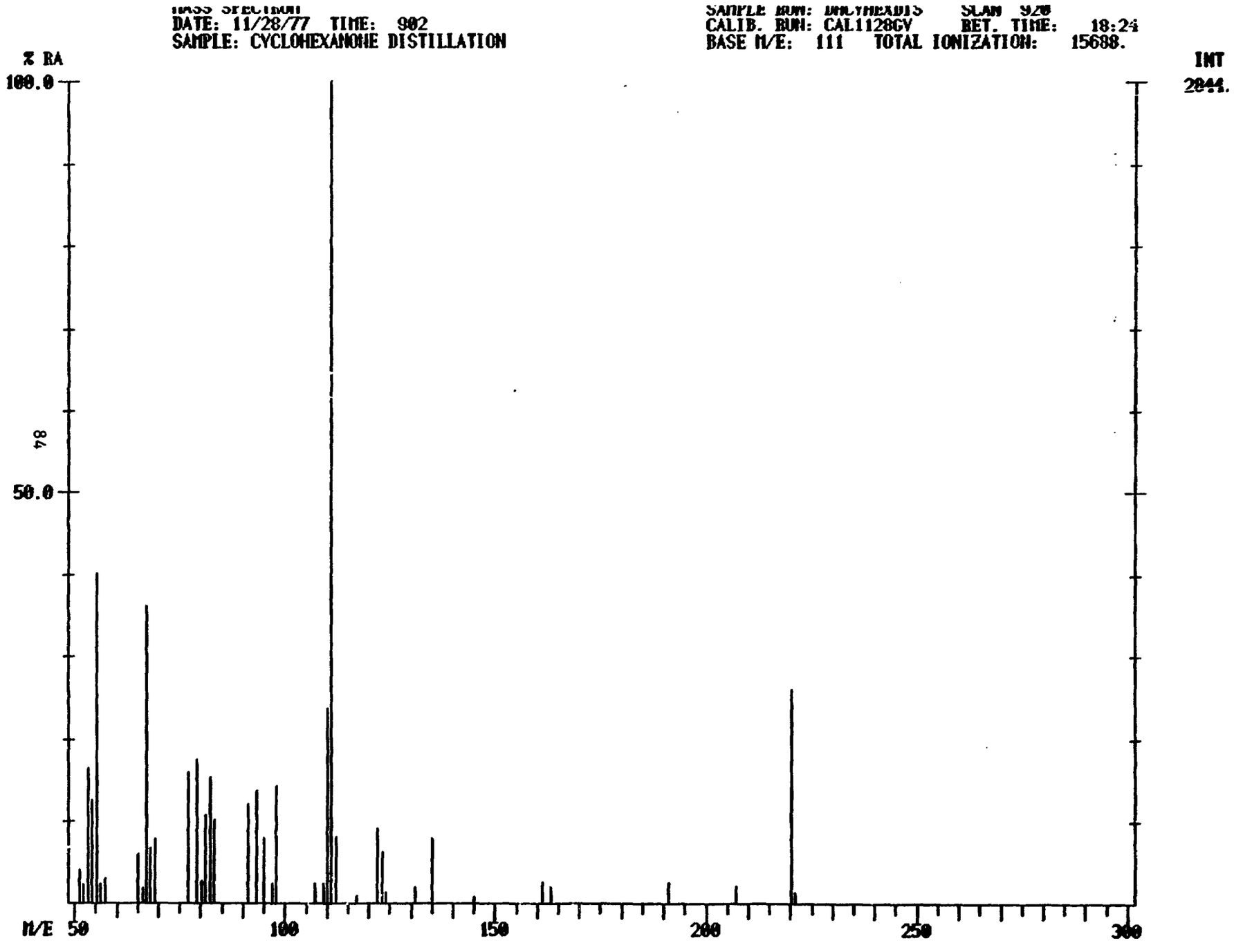


Figure 10 - Scan 920, Bar Graph

LOW RESOLUTION MASSES SAMPLE RUN: DHCYHEXDIS SCAN 920
DATE: 11/28/77 TIME: 902 CALIB. RUN: CAL1128GV RET. TIME: 18:24
SAMPLE: CYCLOHEXANONE DISTILBASE M/E: 111 TOTAL IONIZATION: 15688.
LIST THRESHOLD = 1.00 % RELATIVE ABUNDANCE

PEAK NO.	NOMINAL MASS	% RA	% TI	INT
1	41?	15.75	2.86	448.
2	42?	14.21	2.58	404.
3	43?	13.22	2.40	376.
4	44?	43.88	7.95	1248.
5	51	4.15	0.75	118.
6	52	2.29	0.41	65.
7	53	16.46	2.98	468.
8	54	12.59	2.28	358.
9	55	40.15	7.28	1142.
10	56	2.39	0.43	68.
11	57	3.02	0.55	86.
12	65	6.01	1.09	171.
13	66	1.90	0.34	54.
14	67	36.08	6.54	1026.
15	68	6.72	1.22	191.
16	69	7.81	1.42	222.
17	77	15.96	2.89	454.
18	79	17.44	3.16	495.
19	80	2.78	0.50	79.
20	81	10.83	1.96	308.
21	82	15.37	2.79	437.
22	83	10.13	1.84	288.
23	91	12.06	2.19	343.
24	93	13.75	2.49	391.
25	95	7.88	1.43	224.
26	97	2.46	0.45	70.
27	98	14.28	2.59	406.
28	107	2.50	0.45	71.
29	109	2.39	0.43	68.
30	110	23.77	4.31	676.
31	111	100.00	18.13	2844.
32	112	8.09	1.47	230.
33	117	0.98	0.18	28.
34	122	9.14	1.66	260.
35	123	6.29	1.14	179.
36	124	1.44	0.26	41.
37	131	2.04	0.37	58.
38	135	7.91	1.43	225.
40	161	2.53	0.46	72.
41	163	1.97	0.36	56.
42	191	2.64	0.48	75.
43	207	2.14	0.39	61.
44	220	26.05	4.72	741.
45	221	1.41	0.25	40.

Figure 11 - Scan 920, Peak List

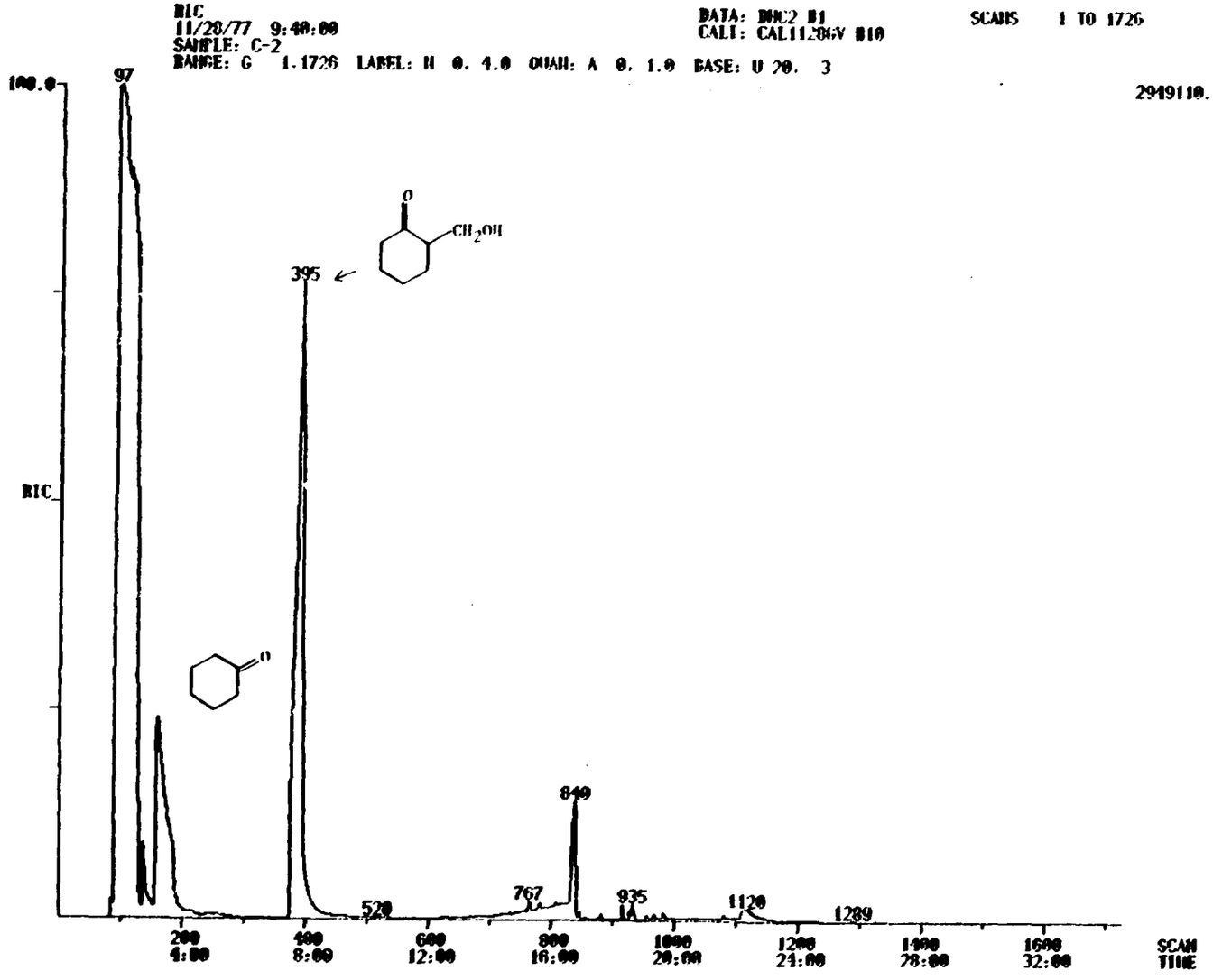


Figure 12 - Sample DHC2

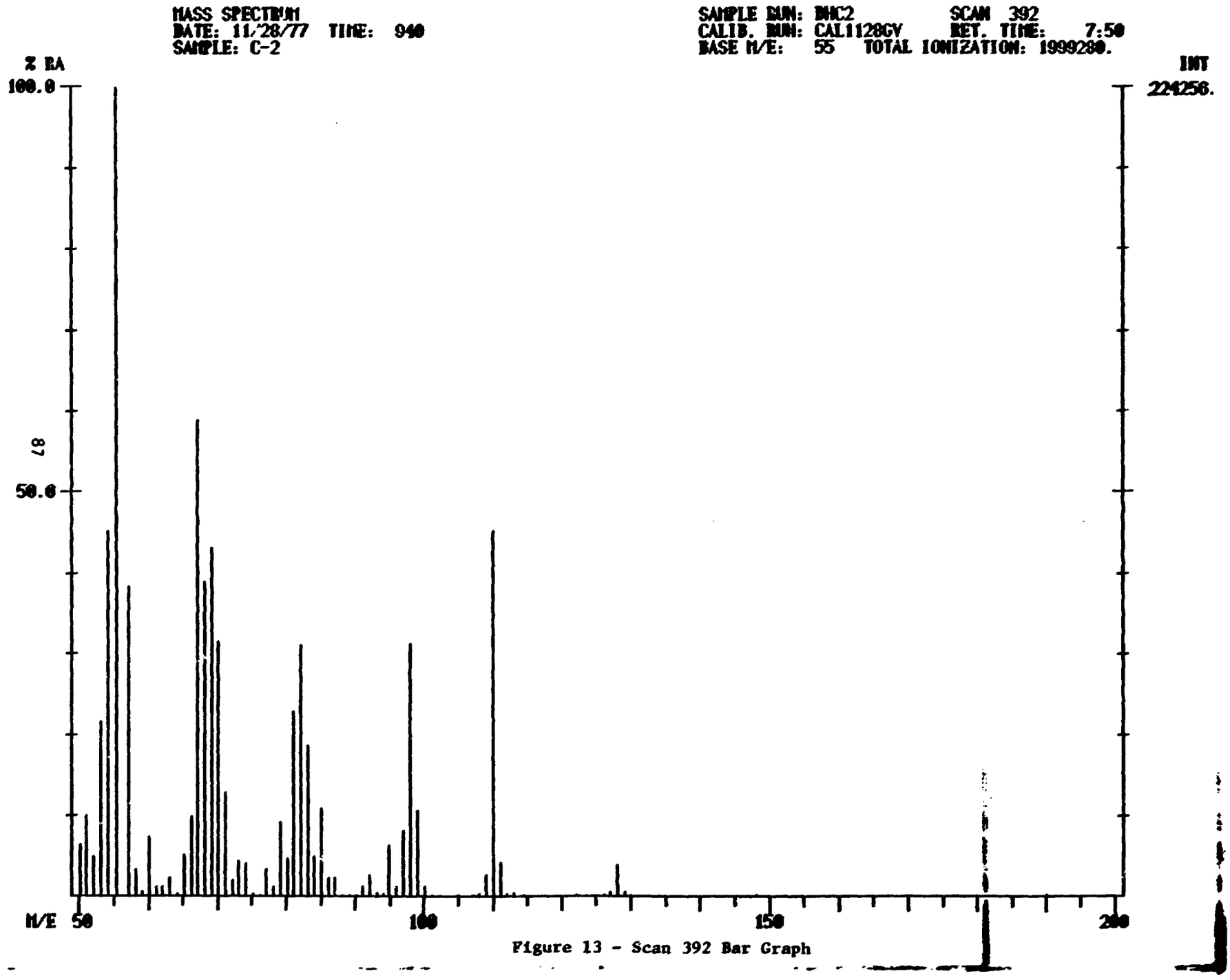


Figure 13 - Scan 392 Bar Graph

LOW RESOLUTION MASSES SAMPLE RUN: DHC2 SCAN 392
DATE: 11/28/77 TIME: 940 CALIB. RUN: CAL1128GV RET. TIME: 7:50
SAMPLE: C-2 BASE M/E: 43 TOTAL IONIZATION: 1999280.
LIST THRESHOLD = 1.00 X RELATIVE ABUNDANCE

PEAK NO.	NOMINAL MASS	% RA	% TI	INT
1	43?	100.00	22.15	442879.
2	45?	2.30	0.51	10207.
7	50	3.26	0.72	14415.
8	51	5.04	1.12	22303.
9	52	2.51	0.56	11103.
10	53	10.97	2.43	48575.
11	54	22.86	5.06	101247.
12	55	50.64	11.22	224255.
13	57	19.45	4.31	86143.
14	58	1.71	0.39	7559.
16	60	3.70	0.82	16383.
19	63	1.13	0.25	5015.
21	65	2.59	0.57	11471.
22	66	4.99	1.11	22111.
23	67	29.77	6.59	131839.
24	68	19.71	4.37	87295.
25	69	21.85	4.84	96767.
26	70	15.98	3.54	70783.
27	71	6.47	1.43	28671.
28	72	1.01	0.22	4487.
29	73	2.21	0.49	9775.
30	74	2.08	0.46	9199.
33	77	1.67	0.37	7399.
35	79	4.61	1.02	20415.
36	80	2.35	0.52	10399.
37	81	11.53	2.55	51071.
38	82	15.75	3.49	69759.
39	83	9.47	2.10	41919.
40	84	2.49	0.55	11023.
41	85	5.53	1.23	24511.
42	86	1.14	0.25	5055.
43	87	1.13	0.25	5023.
48	92	1.32	0.29	5855.
51	95	3.19	0.71	14127.
53	97	4.08	0.90	18047.
54	98	15.78	3.50	69887.
55	99	5.37	1.19	23775.
61	109	1.31	0.29	5815.
62	110	22.99	5.07	101375.
63	111	2.14	0.47	9471.
70	128	1.99	0.44	8831.

Figure 14 - Scan 392, Peak List

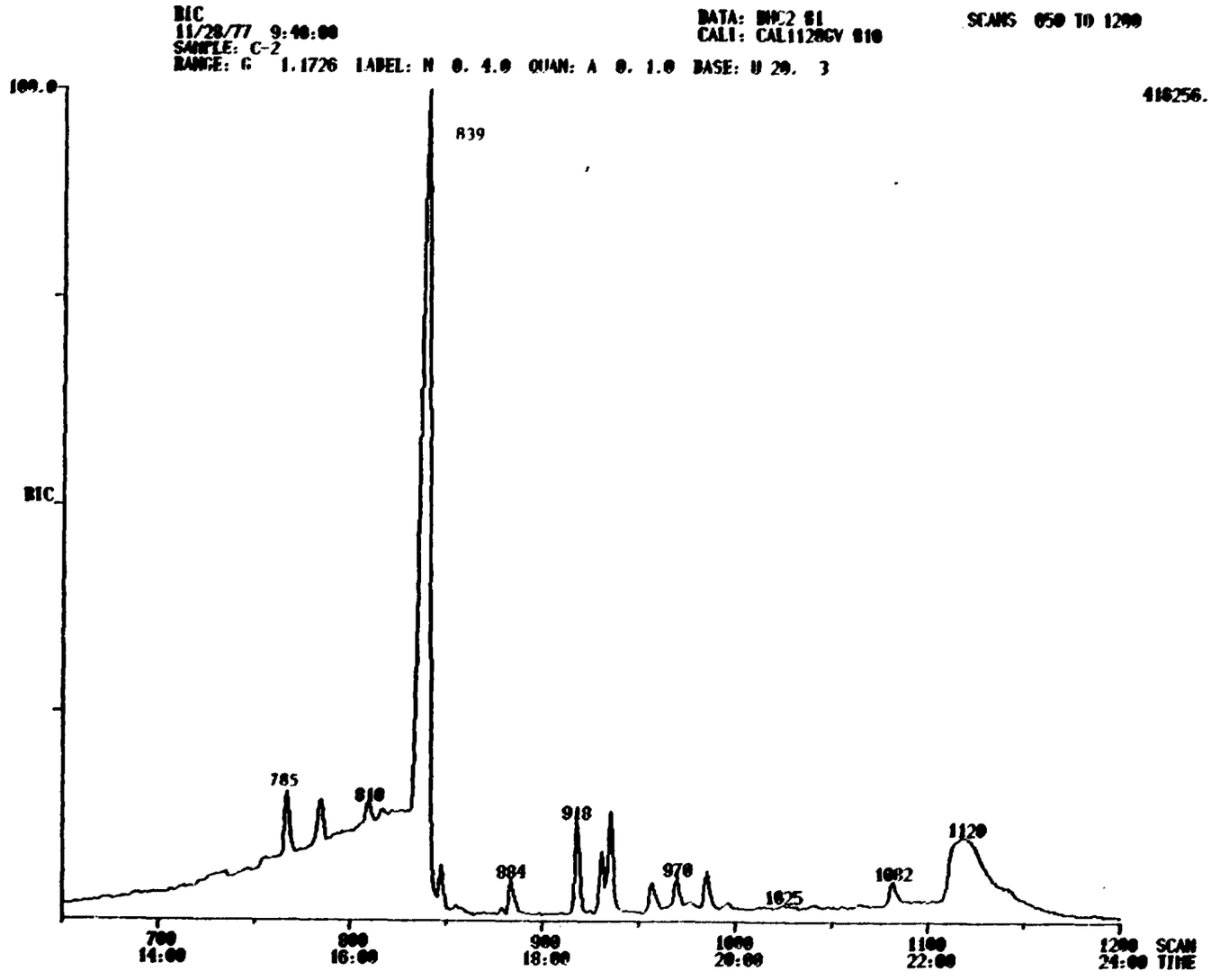


Figure 15 - Sample DHC2, Scan 600-1200

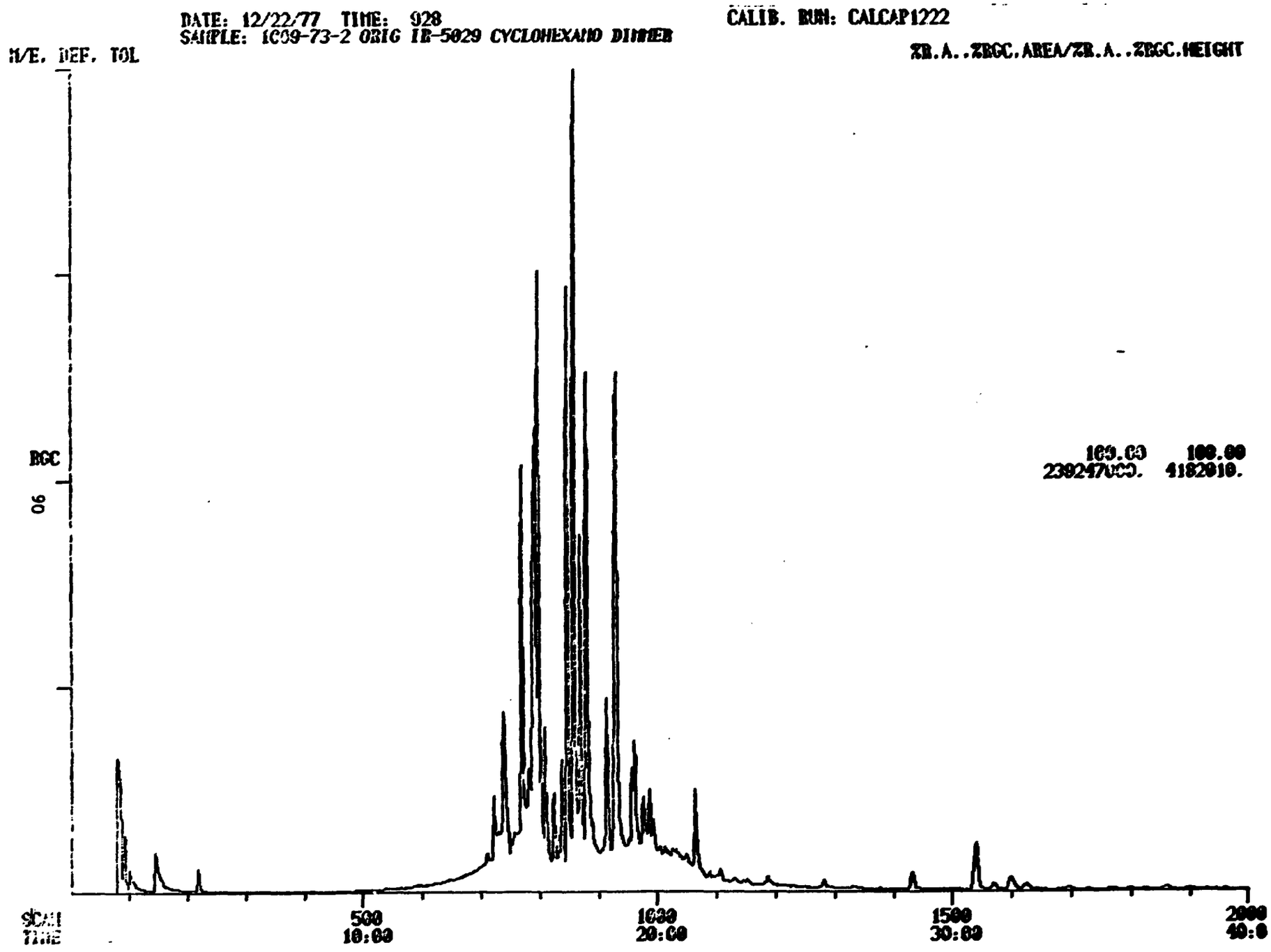


Figure 16 - Sample 1009-73-2

RECONST. GAS CHROMATOGRAM
DATE: 12/22/77 TIME: 928
SAMPLE: 1009-73-2 ORIG IB-5029 CYCLOHEXANO DIMMER

SAMPLE RUN: DH1009732 SCANS 700 TO 1100
CALIB. RUN: CALCAP1222

%R.A..%BGC.AREA/%R.A..%BGC.HEIGHT

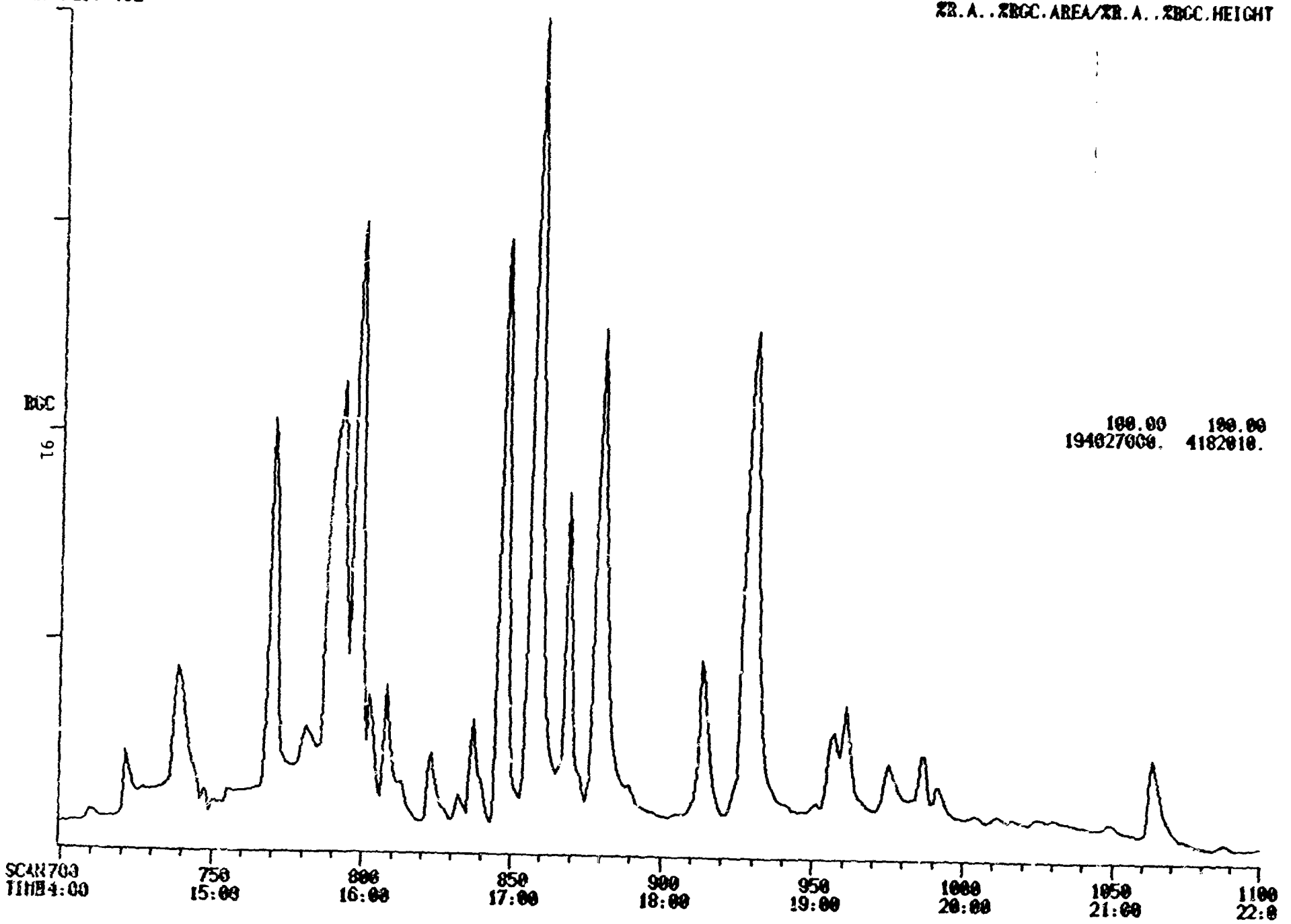


Figure 17 - Sample 1009-73-2, Scan 700-1100

RECONST. GAS CHROMATOGRAM
DATE: 12/22/77 TIME: 928
SAMPLE: 1009-73-2 ORIG IR-5029 CYCLOHEXANO DIMMER

SAMPLE RUN: DH1009/52 SCANS 1100 TO 2000
CALIB. RUN: CALCAP1222

%R.A.,%RGC.AREA/%R.A.,%RGC.HEIGHT

100.00 100.00
22377900. 250367.

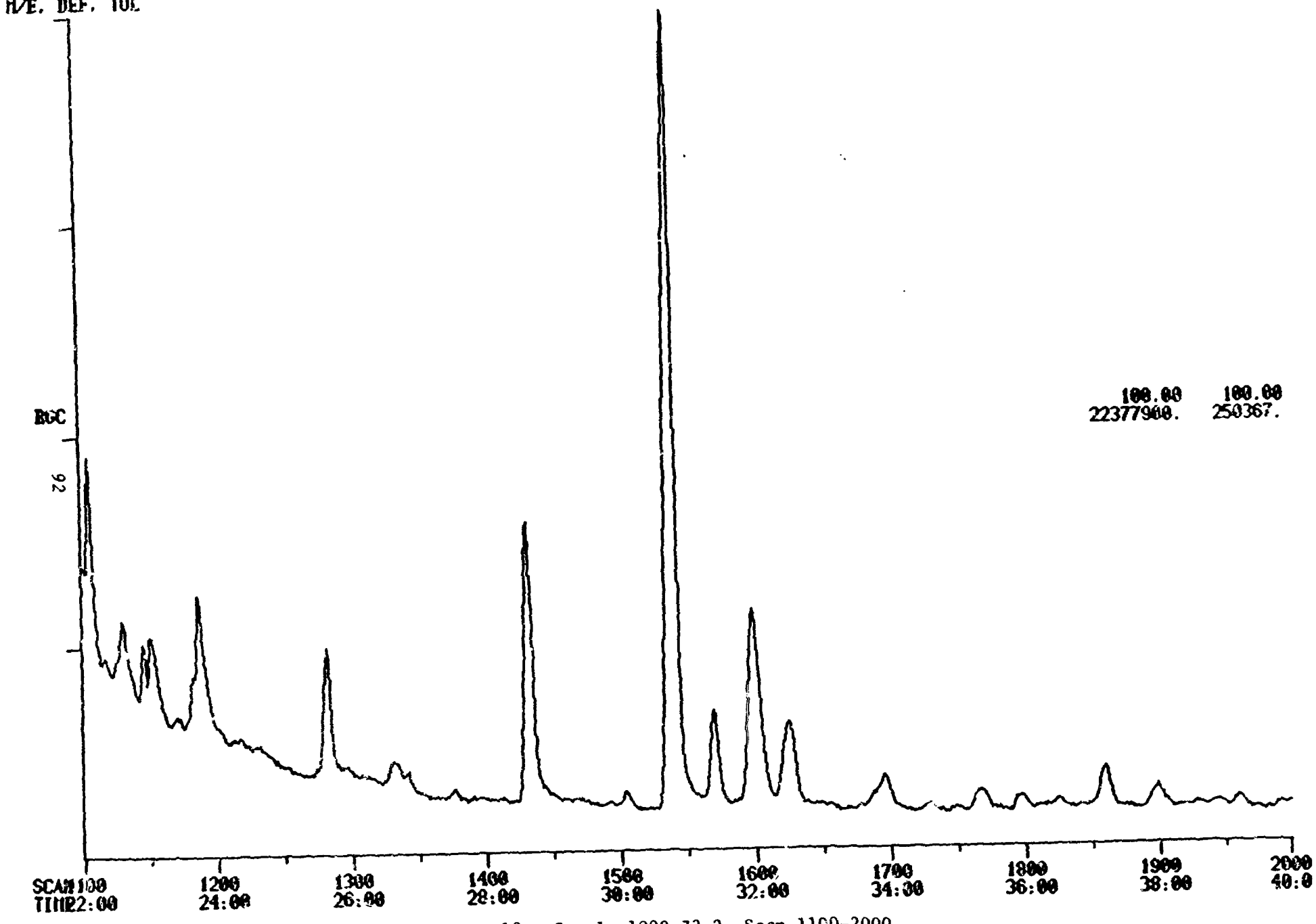


Figure 18 - Sample 1009-73-2, Scan 1100-2000

July 27, 1978

Major Illar Muul
Attn: SGRD-UBG
Environmental Protection Department
U.S. Army Medical Bioengineering
Research and Development Laboratory
Fort Detrick
Frederick, Maryland 21701

Subject: Subtask 9, Identification of Waste Products from RDX and HMX Manufacture, Monthly Report No. 17.

Name of Contractor: Midwest Research Institute
425 Volker Boulevard
Kansas City, Missouri 64110

Contract Number: DAMD-17-74-C-4073 Mod. 708

Program Director: Dr. Cheng-Chun Lee

Phone Number: 816-753-7500

Date of Report: July 27, 1978

Period Covered: January 1 through July 24, 1978

Gentlmen:

Reference samples of impurities found in the cyclohexanone wastes have been synthesized or purchased in gram quantities for structural proof. At the suggestion of Dr. David Rosenblatt these have been shipped to Holston AAP for use in routine monitoring. Examination of a solid residue from extraction of Holston AAP Building H-2 wastewater by capillary GC-MS and high performance liquid chromatography (HPLC) indicated the presence of nitroamines RDX, HMX, TAX and SEX.

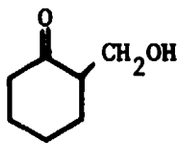
Experimental procedures and future work are discussed below.

Major Illar Muul
U.S. Army Medical Bioengineering
Research and Development Laboratory

I. Experimental Procedures

A. Synthesis and Confirmation of 2-Hydroxymethylcyclohexanone (I)

1. Synthesis of Compound I



I

To a stirred mixture of 49 g (0.5 M) of cyclohexanone, 19 g (0.25 M) of 40% formaldehyde, 30 ml of water and three drops of phenolphthalein solution was added sufficient calcium hydroxide to turn the mixture purple. It was stirred at room temperature for 6 hr, brought to pH 4 with dilute HCl, saturated with ammonium chloride and the organic layer extracted with 4 x 50 ml ether. The extracts were dried, evaporated and the residue distilled to give a first fraction of recovered cyclohexanone followed by 4 g of product, b.p., 100 to 108°/2.5 mm (lit. b.p. 89-91°/5 mm;[1/] b.p. 114 to 115°/16 mm.[2/]

The sample was examined by GC under the following conditions:

a. Instrument: Varian 2400 with flame ionization detector.

b. Column: 6 ft x 4 mm ID, 3% OV-1 on Anakrom A 60/80 mesh.

c. Nitrogen flow: 40 cc/min.

d. Temperatures: Column - Varied from 50 to 170° at 8°/min.

Detector - 200°

Injector - 200°

1/ Zimmerman, H., and J. English, JACS, 76, 2285 (1954).
2/ Mannich, C., and W. Brose, Ber, 56B, 833 (1923).

e. Results: Under all conditions studied only one peak was observed.

A copy of the infrared spectrum is attached (Figure 1).

The elemental analysis was as follows:

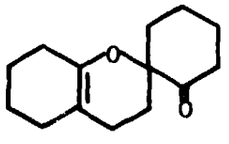
	% C	% H	% O
Theory for $C_7H_{12}O_2$	66.11	8.72	25.16
Observed[3/]	65.67	9.43	25.10
	65.53	9.48	24.9

Based on assay data the sample is 99 ± 1% pure.

2. Confirmation of Compound I in unknown: Coinjection of synthetic I and a chloroform extract of the wastewater from Holston AAP Building H-2 (sample DHC2 as described in Report No. 16) indicated the presence of Compound I in the extract (GC conditions as in 1. above).

Confirmation by retention time comparison had been previously made (Report No. 16) using a capillary GC column coated with SE-30. Also, the mass spectrum of the unknown was consistent with that of Compound I.

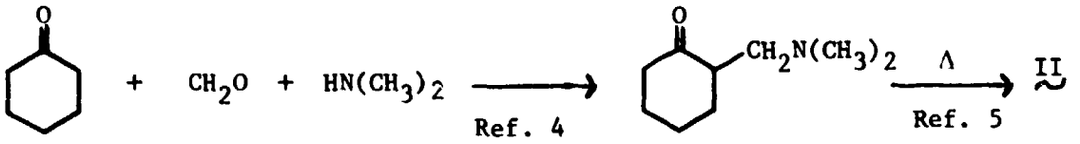
B. Synthesis and Confirmation of Spiro[1-oxocyclohexane-2,2'-3',4',5',6',7',8'-hexahydrobenzo[(b)]pyran] (II)



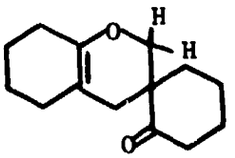
II

3/ Galbraith, Inc., Knoxville, Tennessee.

1. Synthesis of Compound II: Compound II was synthesized according to literature procedures as indicated below:



The infrared spectrum (Figure 2) was consistent with the structure. Further support for the structure was given by NMR (Figure 3). Since the only absorbances were ill-defined multiplets from ~1.8 to 3.1 δ; the synthesized material cannot be structural isomer III. Compound III would show an absorption at ~4 δ for the methylene protons adjacent to the ether oxygen.



III

2. Confirmation of Compound II: Previous work (Report No. 16) has shown that a wastewater component has the same mass as II and its fragmentation pattern is consistent with the structure of II. The synthetic material and wastewater samples DHCYHEXDIS and DHC2 (described in Report No. 16) were examined by GC as described below.

a. Instrument: Varian 2400

b. Column: 6 ft x 4 mm I.D., 3% OV-1 on Anakrom A, 60/80 mesh

c. Nitrogen flow: 40 cc/min

4/ Frank, R., and R. Pierle, J. Am. Chem. Soc., 73, 727 (1951).
5/ Mannich, C., Ber., 74B, 554 (1941).

d. Temperatures: Column - Varied from 130° to 220° at 8°/min

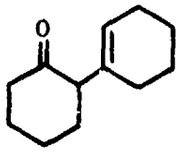
Detector - 250°

Injector - 250°

e. Results: Identical retention times were observed (10.5 min) for the peak in DHCYHEXDIS and DHC2 previously identified as II.

C. Confirmation of 2-(2-Cyclohexenyl)cyclohexanone (III)

A reference sample of III was purchased from Pfaltz and Bauer, Inc. (Stamford, Connecticut) for use in confirming its presence in the wastewater. Examination by GC using the conditions described in B.2. above indicated the presence of III in samples DHCYHEXDIS and DHC2.

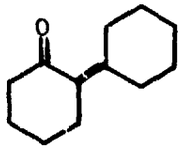


III

A sample of III was shipped to Holston AAP for use in routine monitoring. The exact purity of this sample is being determined.

D. Confirmation of 2-(1-Cyclohexenyl)cyclohexanone (IV)

A reference sample of IV was purchased from Pfaltz and Bauer, Inc. (Stamford, Connecticut) for use in confirming its presence in the wastewaters. This work is underway.



IV

E. Examination of Solid Residue From Wastewater

As described in Monthly Report No. 16 a solid residue was obtained by cooling the chloroform extract of wastewater from Holston AAP Building H-2. Examination of the residue by low resolution GC-MS using a Finnigan 4000 coupled to a 30 meter capillary GC column coated with SE-30 indicated the presence of TAX and RDX as the only components. Examination of the residue by HPLC using a 300 x 3.3 mm column packed with μPak C_{18}® (Waters Associates) with 15% methanol/1% acetic acid in water as eluant, indicated by retention time the presence of TAX, RDX, HMX, SEX and one unknown minor component. The GC and HPLC data are consistent since HMX and SEX will not elute from a GC under the conditions used.

II. Future Work

A sample of 2-(1-cyclohexenyl)cyclohexanone will be supplied to Holston AAP for use in routine monitoring.

At the request of Dr. David Rosenblatt a proposal is being forwarded to assay Holston AAP wastewaters for dimethylnitrosoamine.

Sincerely,

MIDWEST RESEARCH INSTITUTE

Dan Helton

Danny O. Helton
Senior Chemist

Approved:

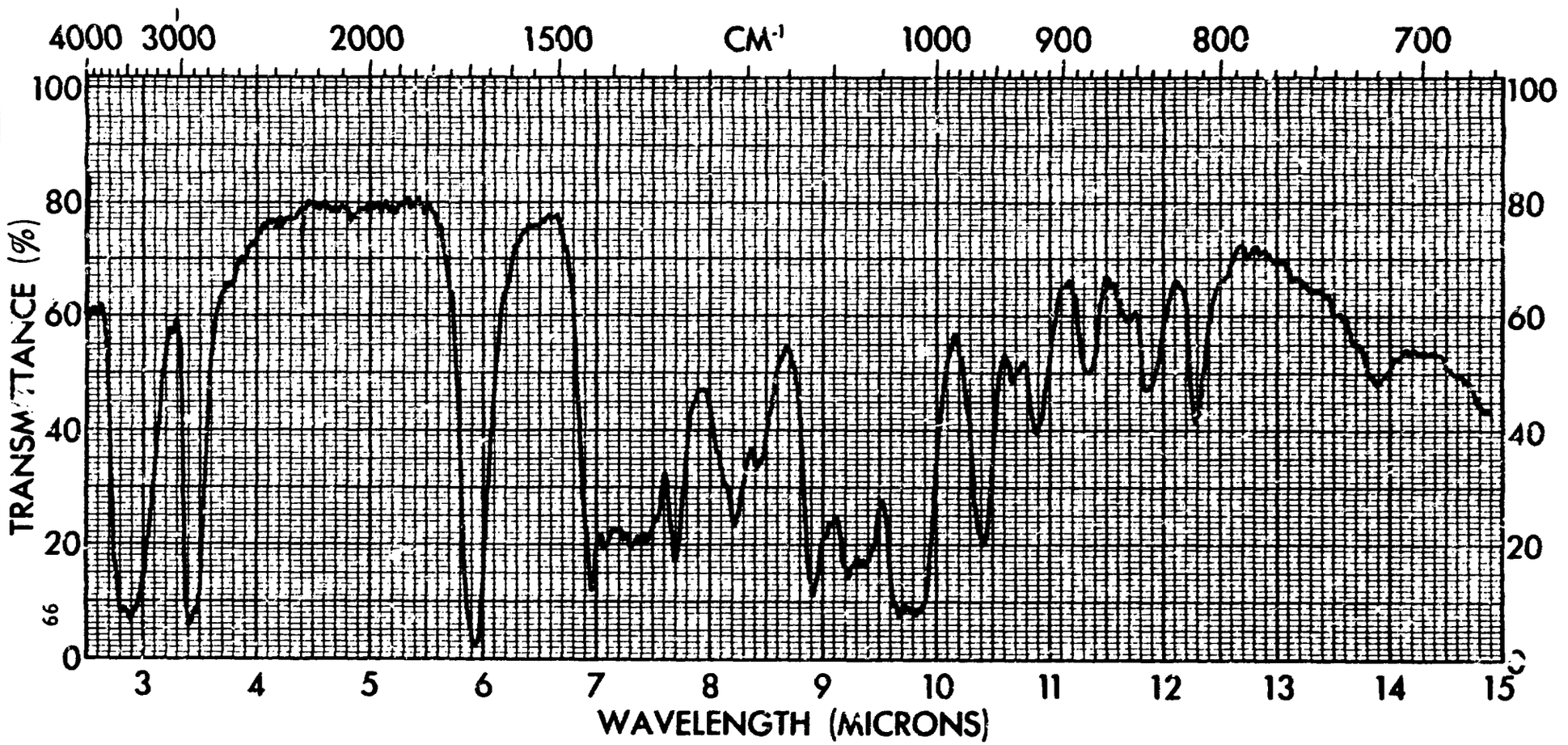
H. M. Hubbard, Acting Director
Biological Sciences Division

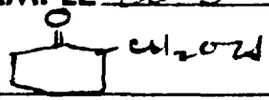
(15 copies of report submitted)

ccs: Ms. Jean Smith
Contract Office
U.S. Army Research and Development Command
Washington, DC 20314

Mr. Russell Jackson
Holston Army Ammunition Plant
Kingsport, Tennessee 37662

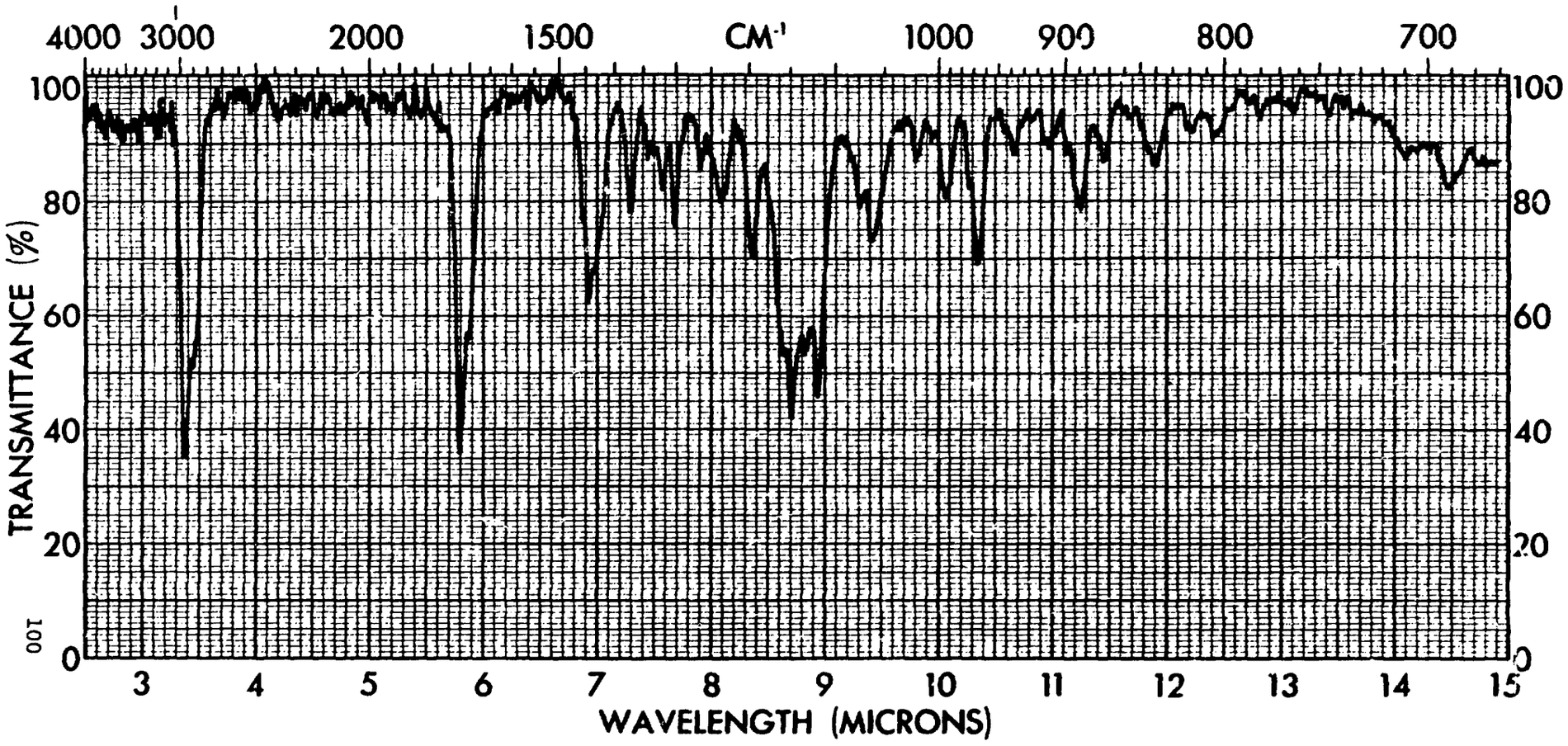
Dr. David Rosenblatt
Environmental Quality Division
U.S. Army Medical Bioengineering Research and Development Laboratory
Fort Detrick
Frederick, Maryland 21701



SPECTRUM NO.	ORIGIN	LEGEND	REMARKS
SAMPLE WB		1.	Figure 1
	PURITY EK 99±1%	2.	2-Hydroxymethylcyclohexanone
	PHASE	DATE	
B.P.	THICKNESS	OPERATOR	

SPECTRUM NO. SAMPLE

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.



SPECTRUM NO.	ORIGIN	LEGEND	REMARKS
SAMPLE WB- 1		1.	Figure 2
	PURITY	2.	Spiro[1-oxocyclohexane-2,2'-3',4',5',6',7',8'-hexahydro-benzo[(b)]pyran]
	PHASE NEAT	DATE 2/24/15	
B. 115-7° /1.5 mm	THICKNESS	OPERATOR WB	

SPECTRUM NO. SAMPLE

PART NO. 137-1281

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.

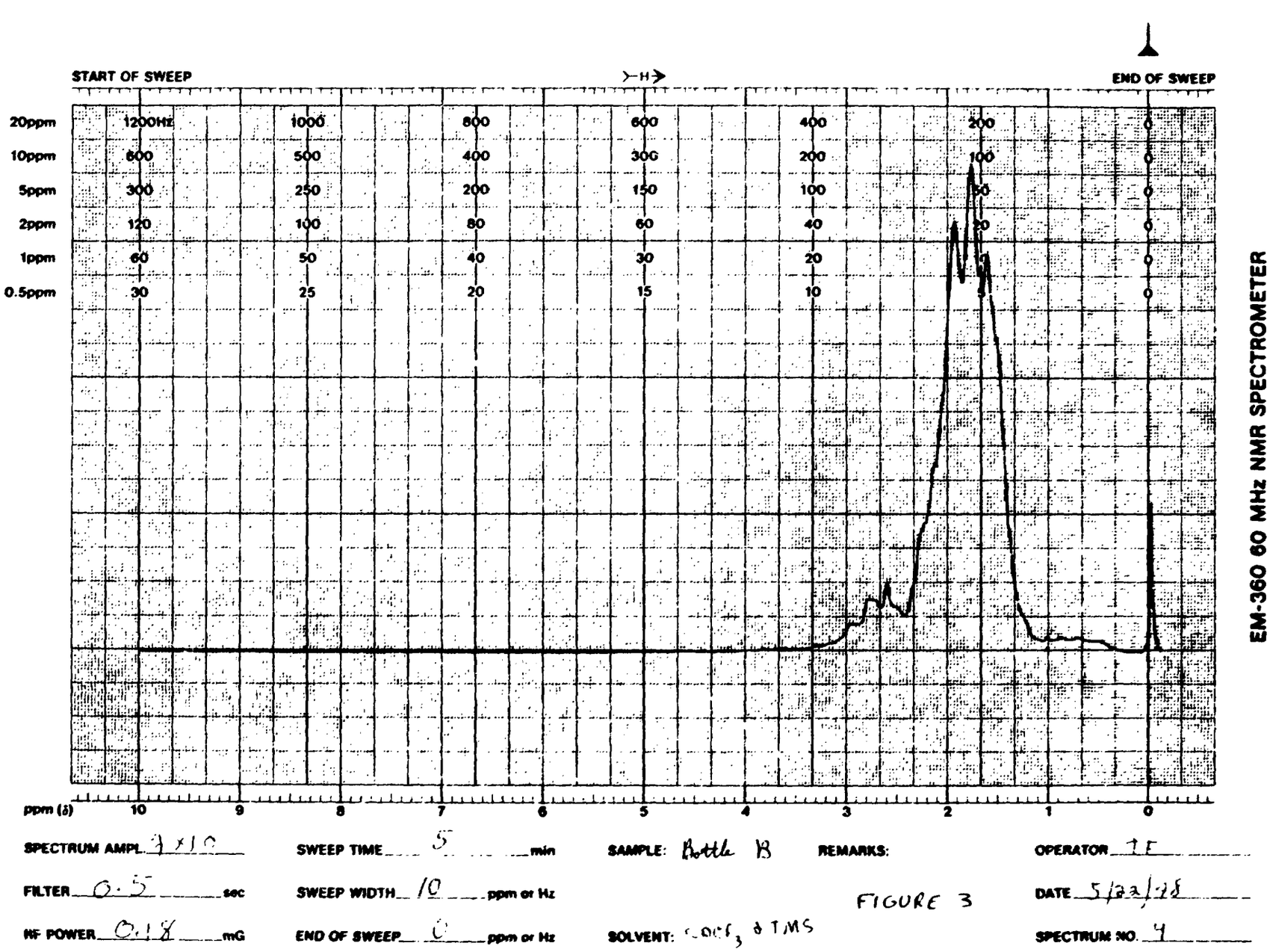


Figure 3

Spiro[1-oxocyclohexane-2,2'-3',4',5',6',7',8'-hexahydrobenzo[(b)]pyran]

APPENDIX E

MONTHLY REPORT NO. 18
IDENTIFICATION AND ASSAY OF DIMETHYLNITROSAMINE

January 9, 1979

Major Illar Muul
Attn: SGRD-UBG
Environmental Protection Department
U.S. Army Medical Bioengineering
Research and Development Laboratory
Fort Detrick
Frederick, Maryland 21701

Subject: MRI Project No. 3900-B, Subtask 9, Identification of Waste Products from RDX and HMX Manufacture, Monthly Report No. 18.

Contract No.: DAMD-17-74-C-4073

Name of Contractor: Midwest Research Institute
425 Volker Boulevard
Kansas City, Missouri 64110

Program Director: Dr. Cheng-Chun Lee

Phone Number: 816-753-7600

Date of Report: January 9, 1979

Period Covered: October 1977 through November 30, 1978

Gentlemen:

Industrial wastewater samples taken at Holston Army Ammunition Plant (HAAP), Kingsport, Tennessee, have been analyzed for the presence of dimethylnitrosamine (DMN). The presence of DMN was indicated by gas chromatography using an alkali flame ionization detector (GC-AFID) and confirmed by use of capillary column gas chromatography-mass spectrometry (GC-MS).

Table 1 lists the DMN concentrations which ranged from 1 to ∿ 500 ppm (weight per volume).

MRI was requested to analyze these samples for DMN because of the possibility that dimethylamine could be formed in HSAAP building A-1 and transformed to DMN by reaction with nitrite in Arnott branch.

Discussed below are Sampling Locations and Procedures (I), Extraction and Gas Chromatographic Analysis (II), Mass Spectral Confirmation (III), Summary and Conclusions (IV), and Suggested Course of Action (V).

Major Illar Muul
U.S. Army Medical Bioengineering
Research and Development Laboratory 2

January 9, 1979

TABLE 1

DMN CONCENTRATIONS IN PARTS PER MILLION

Site 1[a,b]		Site 2[c]		Site 3[c]	
Day 1	Day 3	Day 1	Day 3	Day 1	Day 3
∿ 500 (one analysis)	∿ 500 (one analysis)	0.87 ± 0.03 (two analyses)	1.6 ± 0.1 (two analyses)	6.2 ± 1.0 (four analyses)	11.6 (one analysis)

a/ Site selection is discussed in Section I.
b/ Uncorrected for extraction efficiency, see Section II.B.3.
c/ Corrected for extraction efficiency of 70%.

I. Sampling Locations and Procedures

Site 1 is the effluent pipe from building A-1. Site 2 is Arnott branch about 40 ft downstream on the bank opposite that of the entering A-1 stream. Site 3 is Arnott branch about 500 ft downstream on the bank opposite that of the entering A-1 stream. One-quart grab samples were taken at each location.

Samples were taken on September 13 and 15, placed in glass quart jars, and cooled with Blue Ice® until arrival at Midwest Research Institute. They were then stored at 4°C until extracted on October 21. Prior to use the jars were cleaned with Alconox® and rinsed with distilled water.

II. Extraction and Gas Chromatographic Analysis

The extraction method used is essentially that of W. Fiddler, J. W. Pensabene, R. C. Doerr, and C. J. Dooley, Fd. Cosmet. Toxicol., 15, 441-443 (1977). Details of the extraction and GC-AFID analysis are given below.

A. Extraction

All work was carried out under reduced lighting conditions. One-liter water samples were extracted twice with 400-ml portions of methylene

chloride (Burdick and Jackson Company) using a separatory funnel. The extracts were combined and dried by passage through 70 g of anhydrous sodium sulfate contained in a fritted glass funnel (prewashed with methylene chloride). The methylene chloride was transferred stepwise to a 500-ml Kuderna-Danish evaporative condensor equipped with a 3-ball Snyder column and graduated 10-ml receiver.

The sample volume was reduced to 4 ml using a 70°C water bath followed by concentration to 1.0 ml under a gentle stream of nitrogen. The sample was transferred to screw-capped (Teflon-lined) vials using disposable pipettes prewashed with methylene chloride.

To determine DMN recovery efficiency, samples from Sites 1 and 3 were fortified with 10 and 100 ppm DMN (Aldrich Chemical Company, Lot No. 62-75-9).

B. Gas Chromatographic Analysis

1. Standards: DMN standards were prepared in methylene chloride.

2. Instrumental conditions:

a. Instrument: Perkin-Elmer Model 3920 equipped with alkali flame ionization detector.

b. Column: 0.91 m x 2 mm ID glass, packed with 10% Carbowax 20M + 5% KOH on 100-120 mesh Anakrom AB (supplied by Analabs, Inc., North Haven, Connecticut).

c. Temperatures: oven - 100°C
injector - 130°C
detector - 250°C

d. Detector bead current setting: 515

e. Carrier gas flow: 21 ml/min, He

3. DMN response: DMN had a retention time of 4.6 $\pm$ 0.1 min. Figure 1 indicates detector response was linear up to 500 ng injected.

4. Calculations: The total concentration of DMN (T) in the samples was calculated from the following equation.

$$T = \frac{100 \cdot \frac{\text{final sample volume}}{\text{sample volume injected}} \cdot \frac{\text{sample peak height}}{\text{standard peak height}} \cdot \text{nanograms of standard}}{\text{initial sample volume} \cdot R}$$

where R = recovery fraction = $\frac{\text{total µg in spiked sample} - \text{total µg in unspiked sample}}{\text{µg added}}$

Solving for R at Site 3, the 10 ppm DMN fortification level indicated a recovery of 70%, and the 100 ppm DMN fortification level indicated a recovery of 64%.

5. Results: The results are given in Table 1. The concentration of DMN at Site 1 on Day 1 or 3 can only be approximated because the concentration was high enough to be in the nonlinear range of detector response. Additionally, no fortification levels near this concentration were included; time constraints prevented complete reexamination of the samples.

III. Mass Spectral Confirmation

The presence of DMN was confirmed by capillary column gas chromatography-mass spectrometry (GC-MS).

A. Instrumental Parameters

1. Instrument: Finnigan 4000 with Finnigan GC and Incos data system.

2. Column: 38 m glass capillary, coated with Carbowax 20M.

3. Flow rate: 1 ml He/min (12 psi)

4. Vent time: 6 min

5. Temperatures: column - 50°C for 1 min, then 6°/min to 150°C and hold.

6. Scan time: 1.2 sec

7. Scan range: 18-300 amu

Major Illar Muul
U.S. Army Medical Bioengineering
Research and Development Laboratory 5 January 9, 1979

B. Results

1. DMN standard: Figure 2 is a reconstructed ion chromatogram (RIC) for an injection of 500 ng DMN. The maximum at scan 136 is due to solvent. The 293 maximum is due to the vacuum pump vent being closed. The 475 maximum is due to DMN. Figure 3 shows a bar plot of scan 478 with background correction from scan 485. The dots above some peaks in the bar plot indicate these peaks are found in the background. Although scan 475 is the DMN maximum, scan 478 was chosen for presentation since an injection of 500 ng saturated the detector at scan 475. Figure 4 gives a mass peak list for scan 478. Figure 5 shows an RIC for a limited number of scans around scan 475. Figure 6 shows an extracted ion current plot (EICP) for the four most intense ions from DMN ionization, i.e., m/e 30, 42, 43 and 74. For m/e 42 and 74 the observed intensity saturated the detector, causing a dip in the EICP around scan 475.

2. Site 1, Day 1: Figure 7 is the RIC for the sample extract. Figure 8 is the RIC for a limited number of scans around scan 485. Figure 9 shows an EICP for specific ions m/e 30, 42, 43 and 74 versus scan number. Again the detector is saturated at m/e 42 and 74, causing a dip in the EICP. Figure 10 is a bar plot for scan 479 with background correction from scan 475. Figure 11 is a mass peak list for scan 479. A comparison of the bar plot (Figure 10) and mass peak list (Figure 11) for this sample with the same type of data for the DMN standard (Figures 3 and 4) indicates these are essentially identical and therefore verifies the presence of DMN. The retention time of the standard and sample peaks also match within experimental error, thus providing supportive data for the presence of DMN. Spectrum 479 corrected for background was submitted to the 25,409 mass spectral library file to determine the best fit for these data. Figure 12 indicates an excellent fit for DMN and the absence of coeluting peaks.

3. Site 1, Day 3: The results from Site 1, Day 3, were essentially identical to those of Site 1, Day 1, with regard to DMN identification. Figure 13 is the RIC and Figure 14 is the EICP for m/e 30, 42, 43 and 74.

4. Site 2, Day 3: Figure 15 is the RIC. Figure 16 is the EICP for m/e 30, 42, 43 and 74. Figure 17 is the bar plot for scan 487, and Figure 18 gives the results of the library search.

5. Site 3, Day 3: The results from Site 3, Day 3, were essentially identical to those of Site 1, Day 1, with regard to DMN identification. Figure 19 gives the RIC and Figure 20 gives the EICP for ions 30, 42, 43 and 74.

Major Illar Muul
U.S. Army Medical Bioengineering
Research and Development Laboratory 6 January 9, 1979

IV. Summary and Conclusions

DMN was found in high concentrations (∿ 500 ppm or ∿ 0.05%) in the waste stream from building A-1 at HSAAP and in lower concentrations (∿ 1 to ∿ 12 ppm) in Arnott branch. Comparison of the DMN levels at Sites 2 and 3 on Days 1 and 3 indicated the DMN output level was variable by a factor of ∿ 1.8.

Russell Jackson of HSAAP indicated by telephone on January 2, 1978, that the output from building A-1 was about 43,000 gal/day and the flow in Arnott branch is about 20,000,000 gal/day. If one assumes the A-1 water contains 0.05% DMN, then ∿ 20 gal. DMN is discharged per day and the DMN content in Arnott branch would be ∿ 1 ppm if uniformly distributed. Fortunately, building A-1 operates for only about 1 week every 6 months.

V. Suggested Course of Action

The ultimate source of DMN should be identified. Appropriate measures can then be taken to destroy the DMN or prevent formation. Both water and air sampling studies should be conducted since DMN is somewhat volatile (b.p. 154°C) and the discharge temperature is ∿ 50°C.

Sincerely,

MIDWEST RESEARCH INSTITUTE

Danny O. Helton

Danny O. Helton
Senior Chemist

Approved:

James L. Spigarelli

James L. Spigarelli, Associate Director
Chemical Sciences Division

C. C. Lee

C. C. Lee, Deputy Director
Biological Sciences Division

Major Illar Muul
U.S. Army Medical Bioengineering
Research and Development Laboratory 7

January 9, 1979

cc: Ms. Jean Smith
Contract Office
U.S. Army Research and
Development Command
Washington, D.C. 20314

Dr. David Rosenblatt
Environmental Quality Division
U.S. Army Medical Bioengineering
Research and Development Laboratory
Fort Detrick
Frederick, MD 21701

Mr. Russell Jackson
Holston Army Ammunition Plant
Kingsport, TN 37622

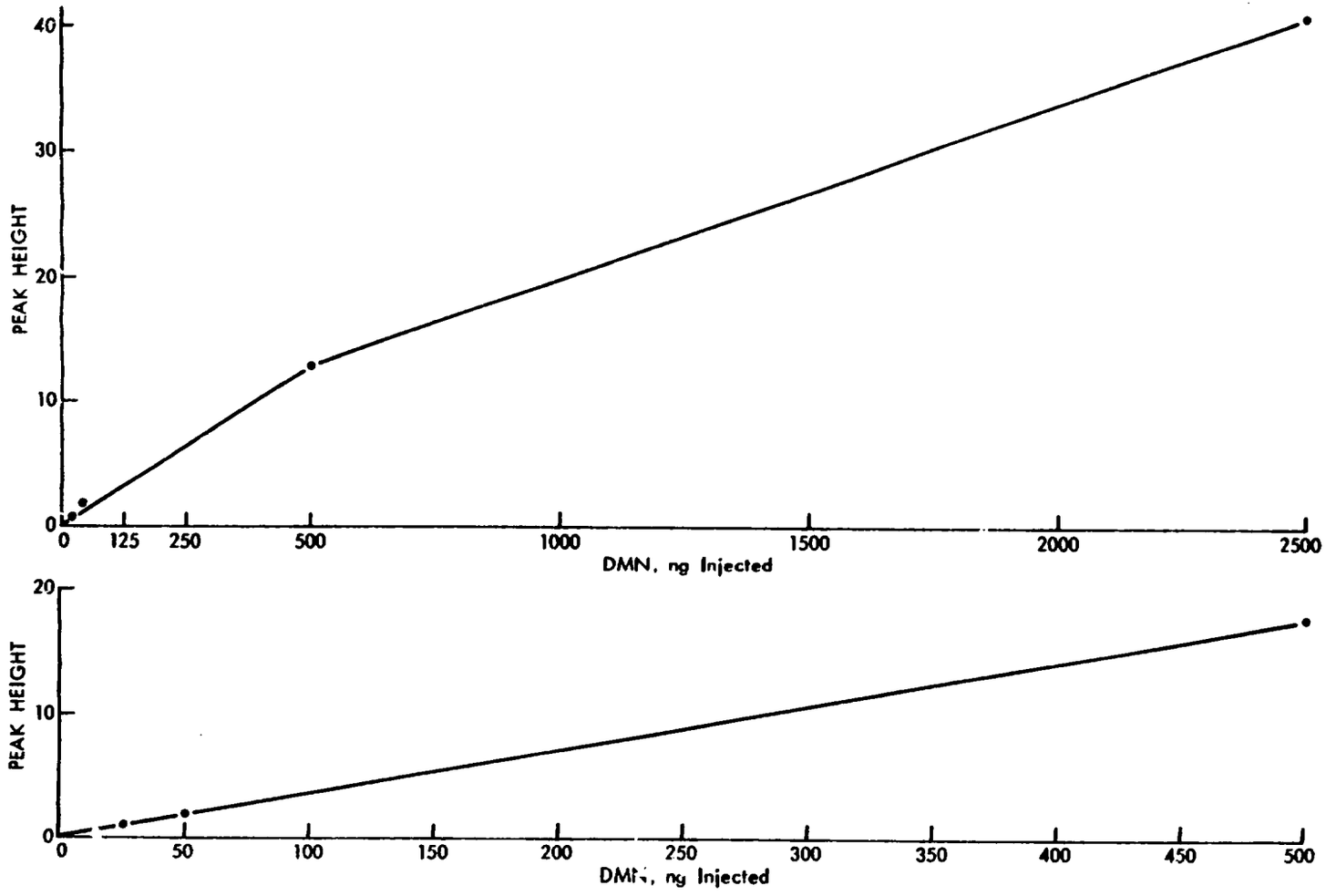


Figure 1 - ng DMN versus Peak Height

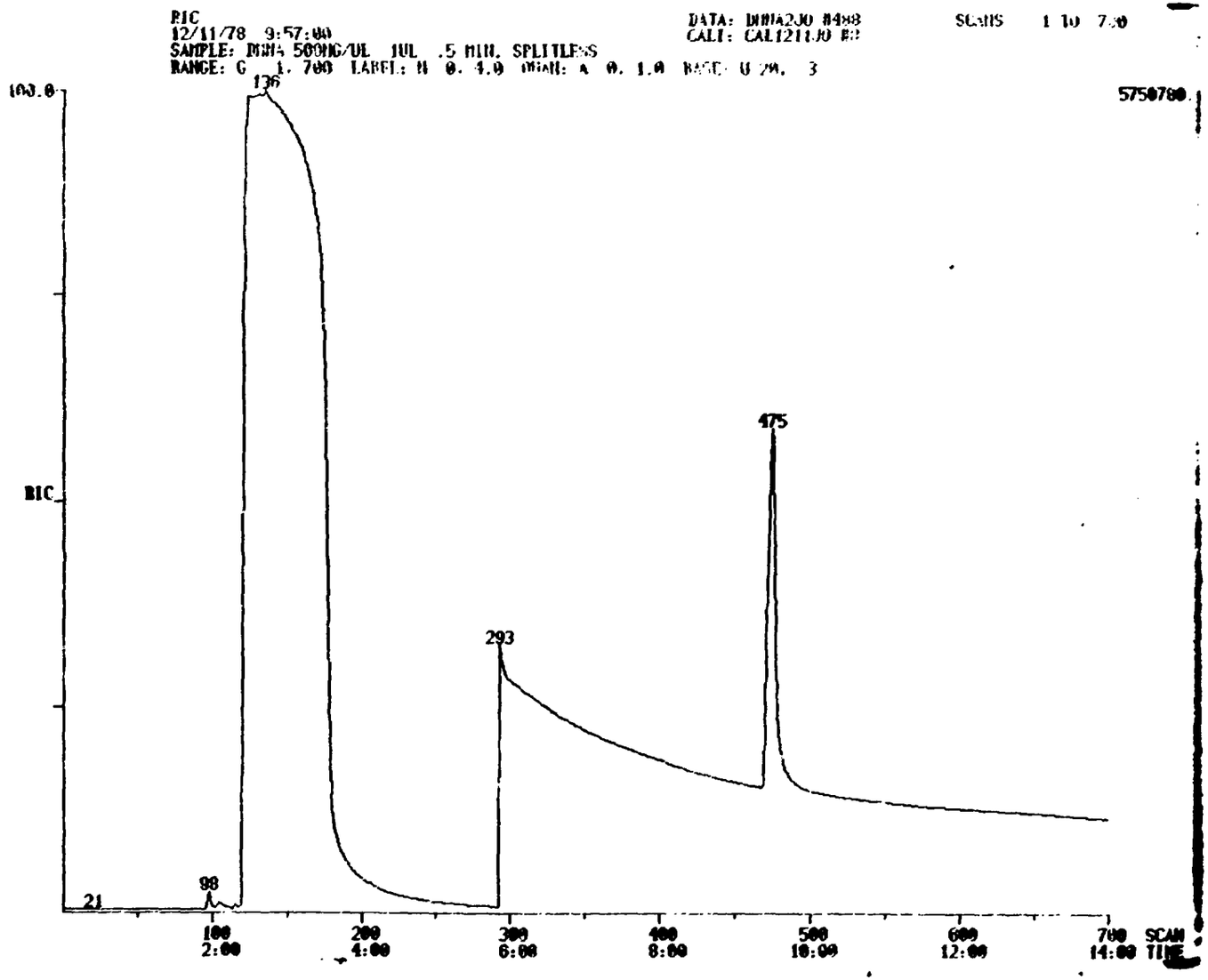


Figure 2 - DMN Standard, Reconstructed Ion Chromatogram (RIC)

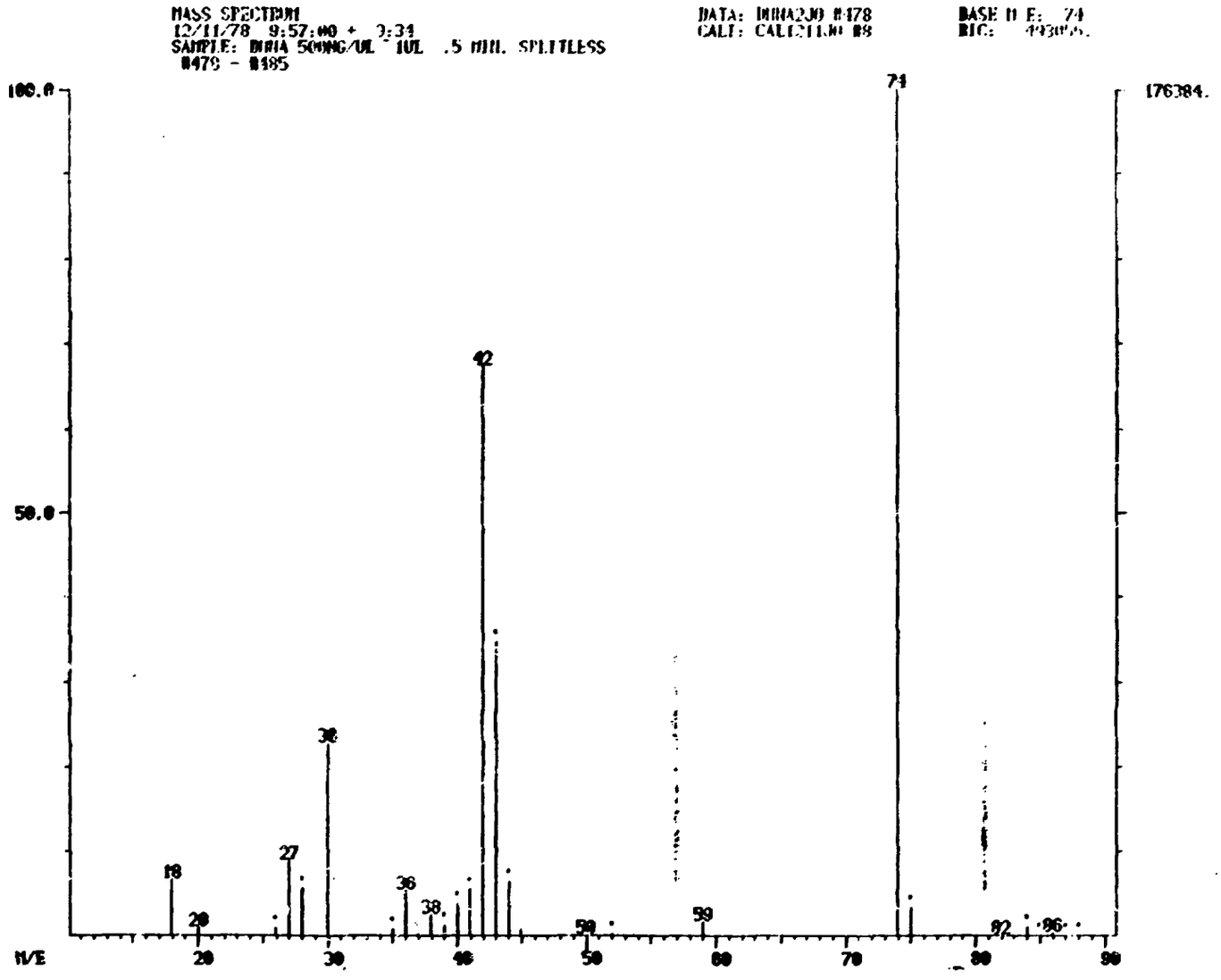


Figure 3 - DMN Standard, Bar Plot

MASS ...I DATA: DMNA2JO # 478 BASE M/E: 74
12/11/70 9:57:00 + 9:34 CALI: CAL1211JO # 8 RIC: 493056.
SAMPLE: DMNA 500NG/UL 1UL .5 MIN. SPLITLESS
#478 - #485

13		0.00	0.00	0.	MINIMA	MIN INTEN:	0.
88				# 0	MAXIMA		
MASS		% BA	% RIC	INTEN.			
16.007	S	6.49	2.32	11440.			
20.00	S	0.91	0.32	1600.			
26.00	S	0.87	0.31	1528.			
27.00		6.83	3.16	15560.			
28.00	S	5.52	1.97	9726.			
30.00	S	22.68	8.11	40000.			
35.00	S	0.78	0.28	1370.			
36.00	S	5.24	1.88	9248.			
37.00	S	0.18	0.07	324.			
38.00	S	2.44	0.87	4304.			
39.00	S	1.34	0.48	2356.			
40.00	S	3.63	1.30	6400.			
41.00	S	5.34	1.91	9424.			
42.00	S	67.34	24.09	118784.			
43.00	S	34.72	12.42	61248.			
44.00	S	6.39	2.28	11264.			
45.00		0.75	0.27	1330.			
50.00	S	0.06	0.02	104.			
52.00	S	0.11	0.04	196.			
59.00	S	1.54	0.55	2712.			
74.00	S	100.00	35.77	176384.			
75.00	S	3.22	1.15	5672.			
82.00	S	0.07	0.02	117.			
84.00	S	0.94	0.34	1664.			
85.00	S	0.01	0.00	12.			
86.00	S	0.22	0.08	384.			
87.00	S	0.04	0.01	69.			
88.00	S	0.05	0.02	96.			

Figure 4 - DMN Standard, Mass Peak List

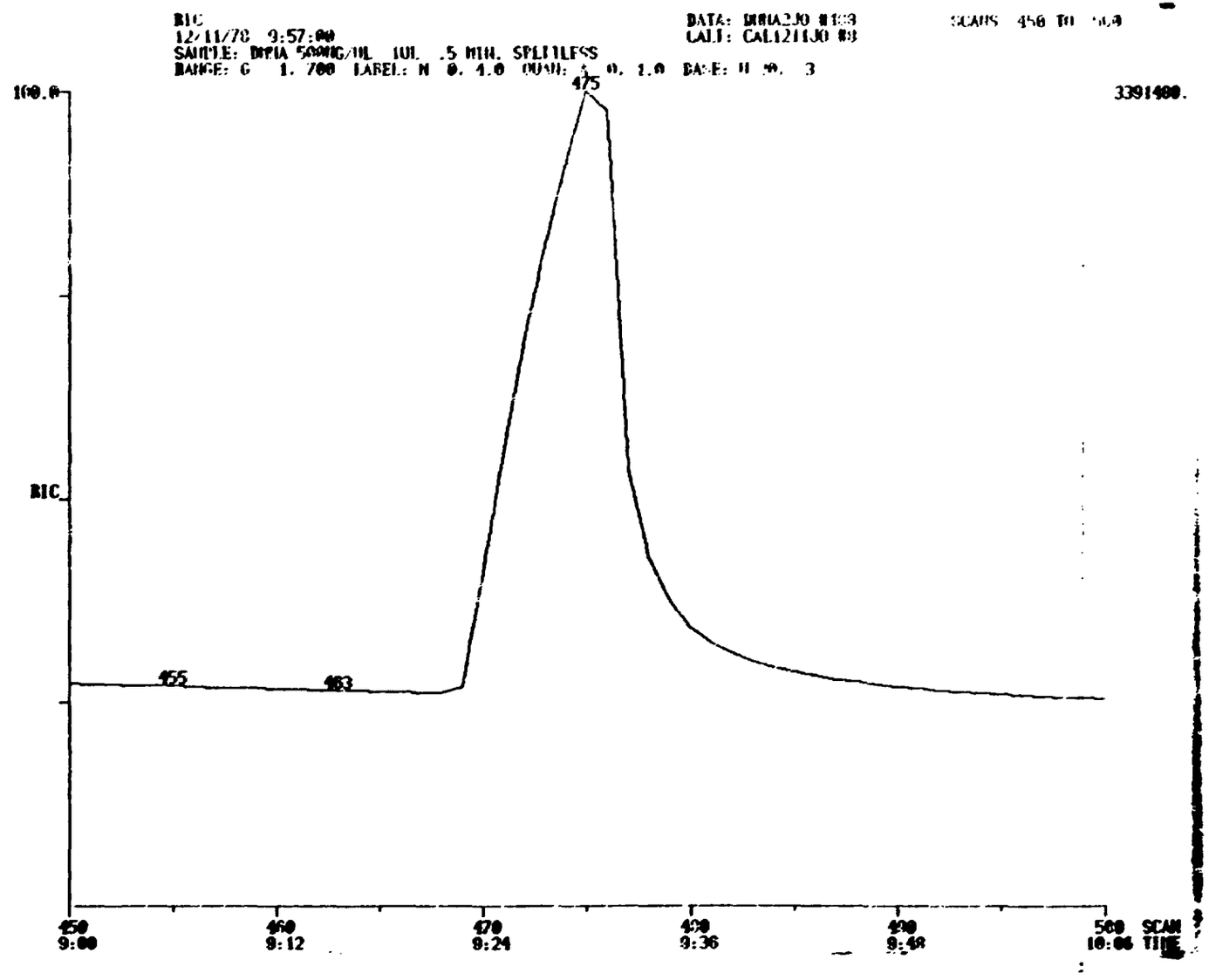


Figure 5 - DMN Standard, RIC for a Limited Number of Scans Around Scan 475

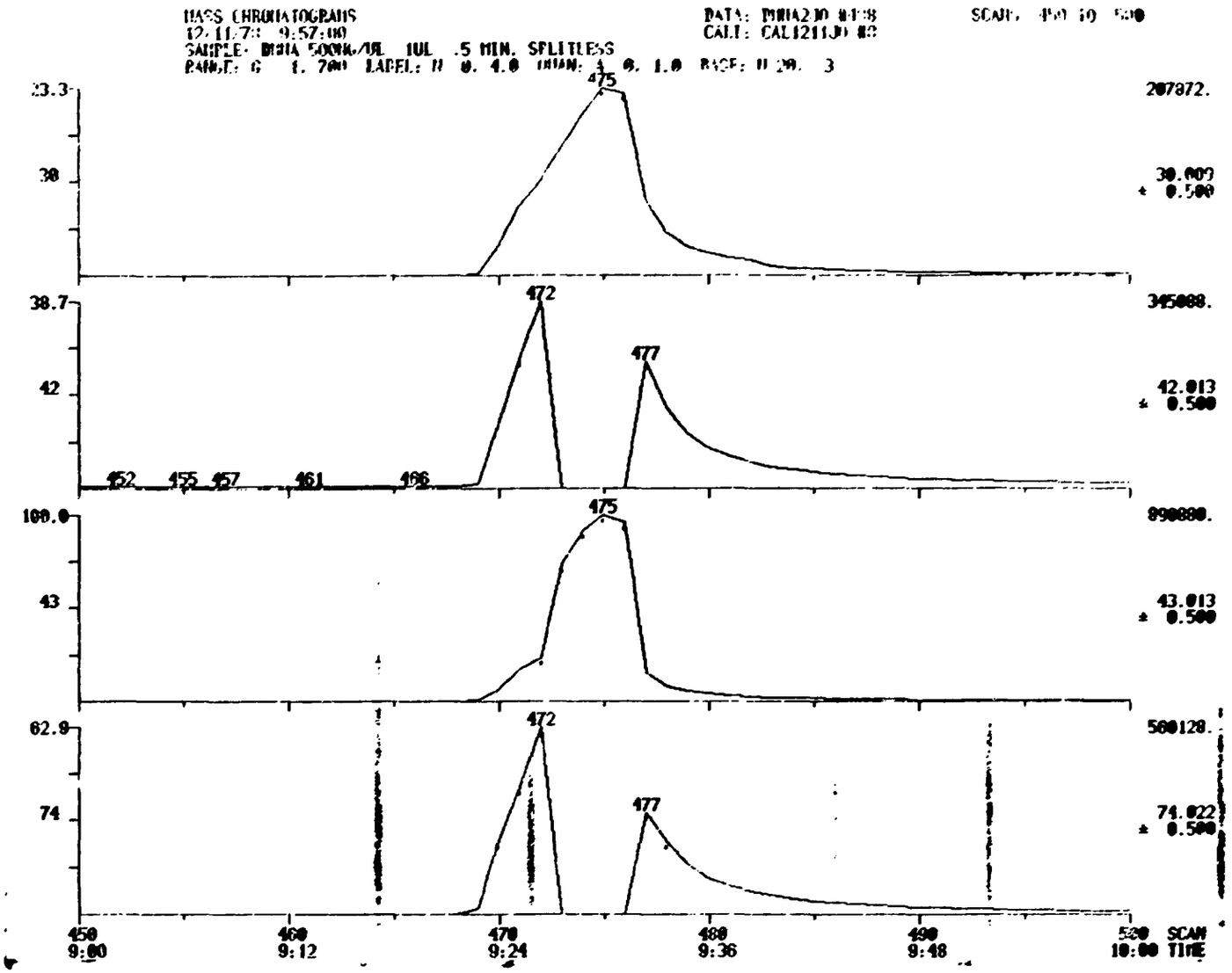


Figure 6 - DMN Standard, Extracted Ion Current Plot (EICP) for m/e 30, 42, 43, and 74

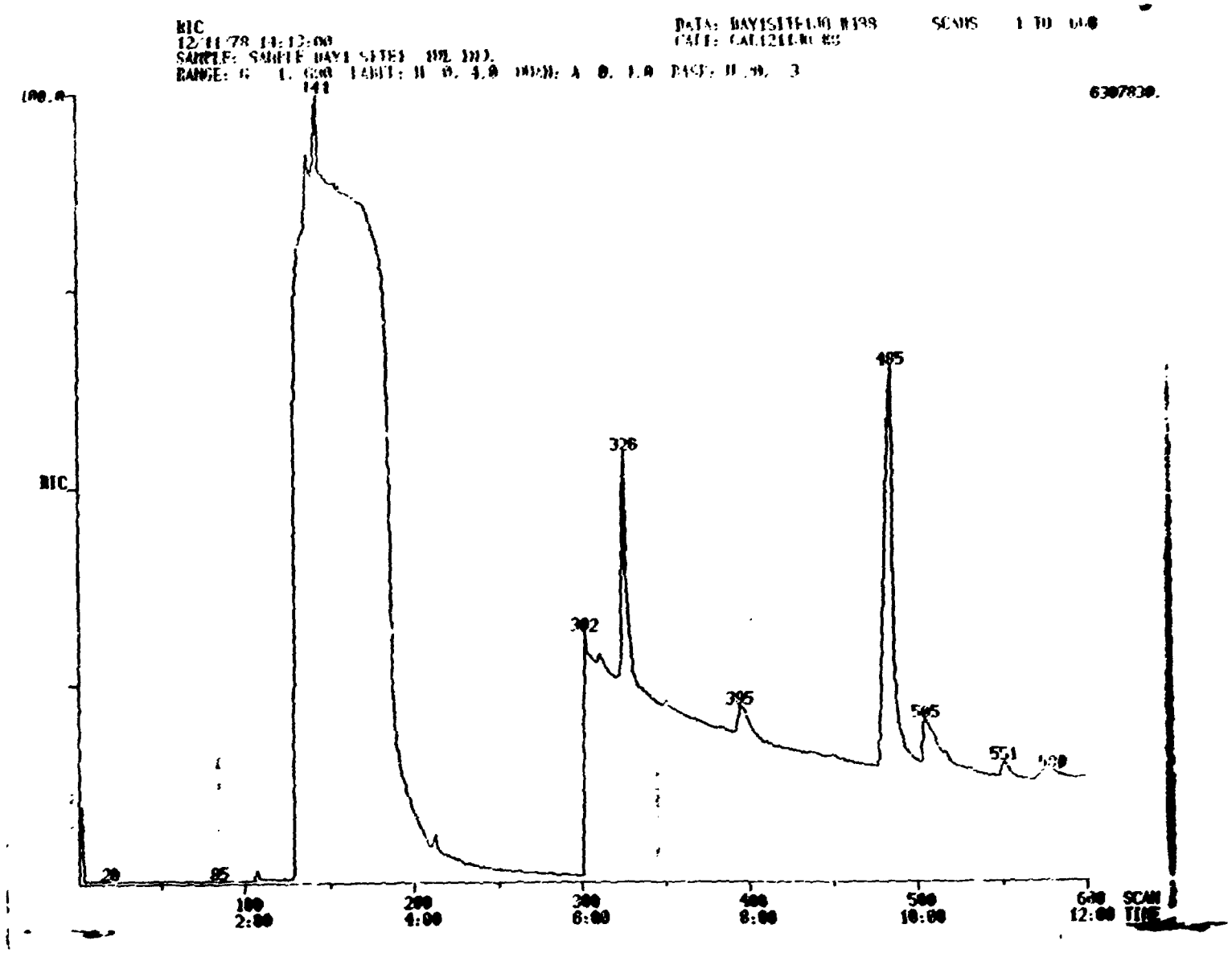


Figure 7 - Site 1, Day 1, RIC

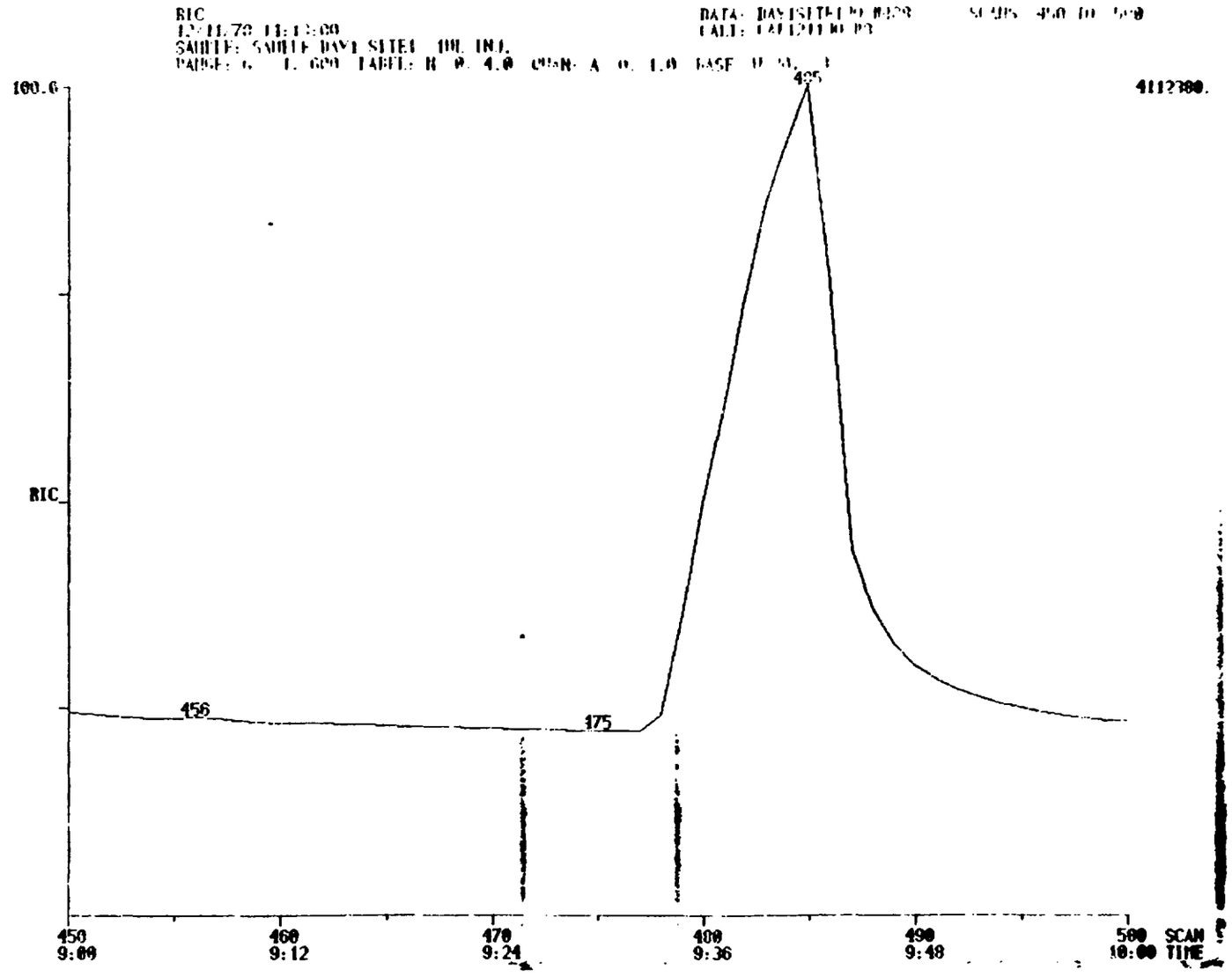


Figure 8 - Site 1, Day 1, RIC for a Limited Number of Scans Around Scan 484

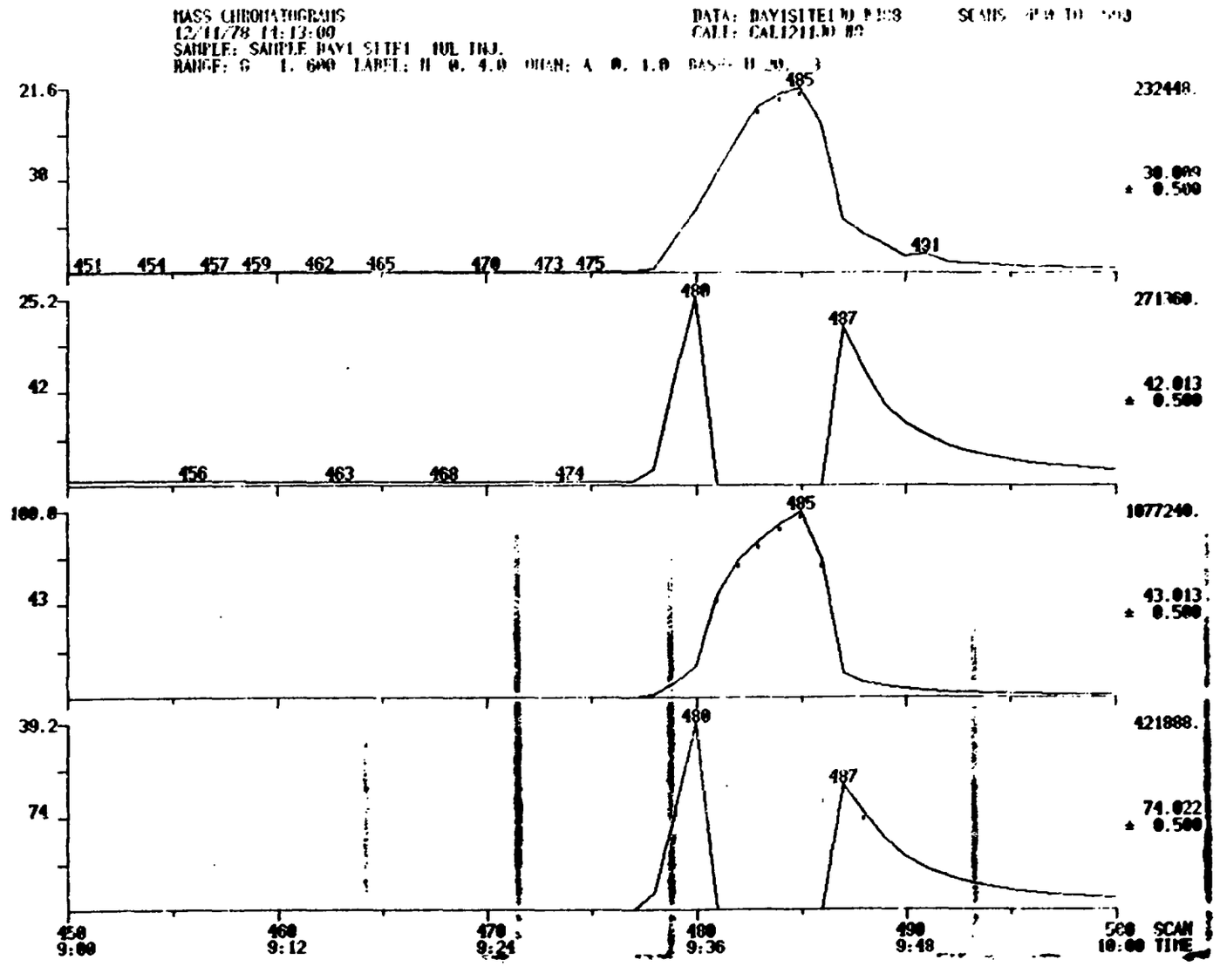


Figure 9 - Site 1, Day 1, EICP for m/e 30, 42, 43 and 74

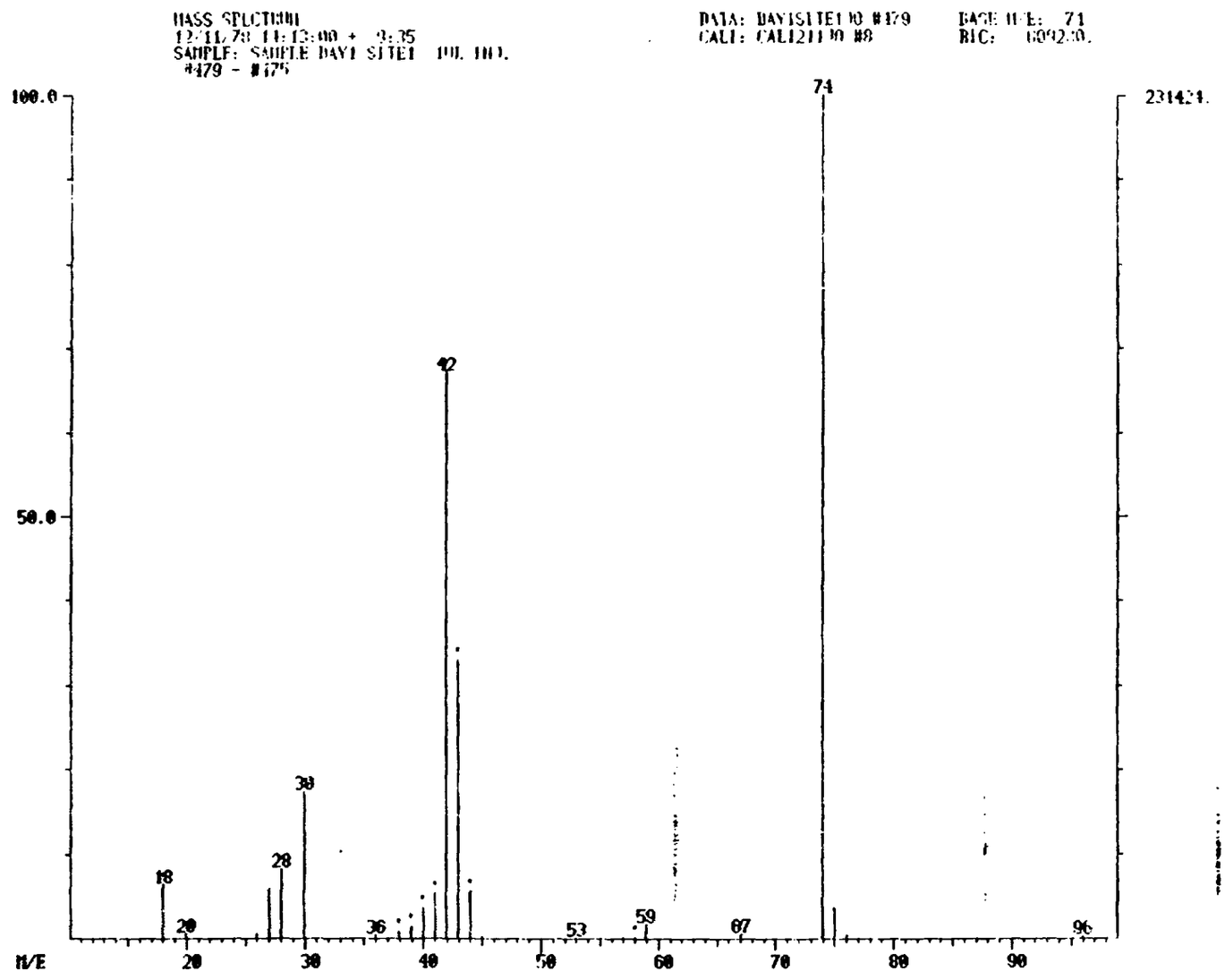


Figure 10 - Site 1, Day 1, Bar Plot

MASS LIST DATA: DAYISITE1JO ● 479 BASE M/E: 74
12/11/78 14:13:00 + 9:35 CALI: CAL1211JO ● 8 RIC: 689280.
SAMPLE: SAMPLE DAY1 SITE1 1UL INJ.
●479 - ●475

18 0.00 0.00 0. MINIMA MIN INTEN: 0.
96 ● 0 MAXIMA

MASS		% RA	% RIC	INTEN.
18.00?	S	6.46	2.45	14944.
20.00	S	0.50	0.19	1152.
26.00		0.56	0.21	1294.
27.00		5.86	2.22	13552.
28.00	S	8.19	3.11	18944.
30.00	S	17.37	6.60	40192.
36.00	S	0.40	0.15	928.
38.00	S	0.92	0.35	2136.
39.00	S	1.44	0.55	3340.
40.00	S	3.49	1.33	8080.
41.00	S	5.27	2.00	12192.
42.00	S	67.15	25.50	155392.
43.00	S	32.96	12.52	76268.
44.00	S	5.51	2.09	12752.
45.00		0.33	0.13	764.
53.00		0.09	0.04	214.
57.00		0.08	0.03	178.
58.00	S	0.10	0.04	231.
59.00		1.70	0.64	3924.
67.00	S	0.53	0.20	1220.
74.00		100.00	37.98	231424.
75.00		3.52	1.34	8144.
76.00		0.49	0.19	1142.
96.00		0.27	0.10	625.

Figure 11 - Site 1, Day 1, Mass Peak List

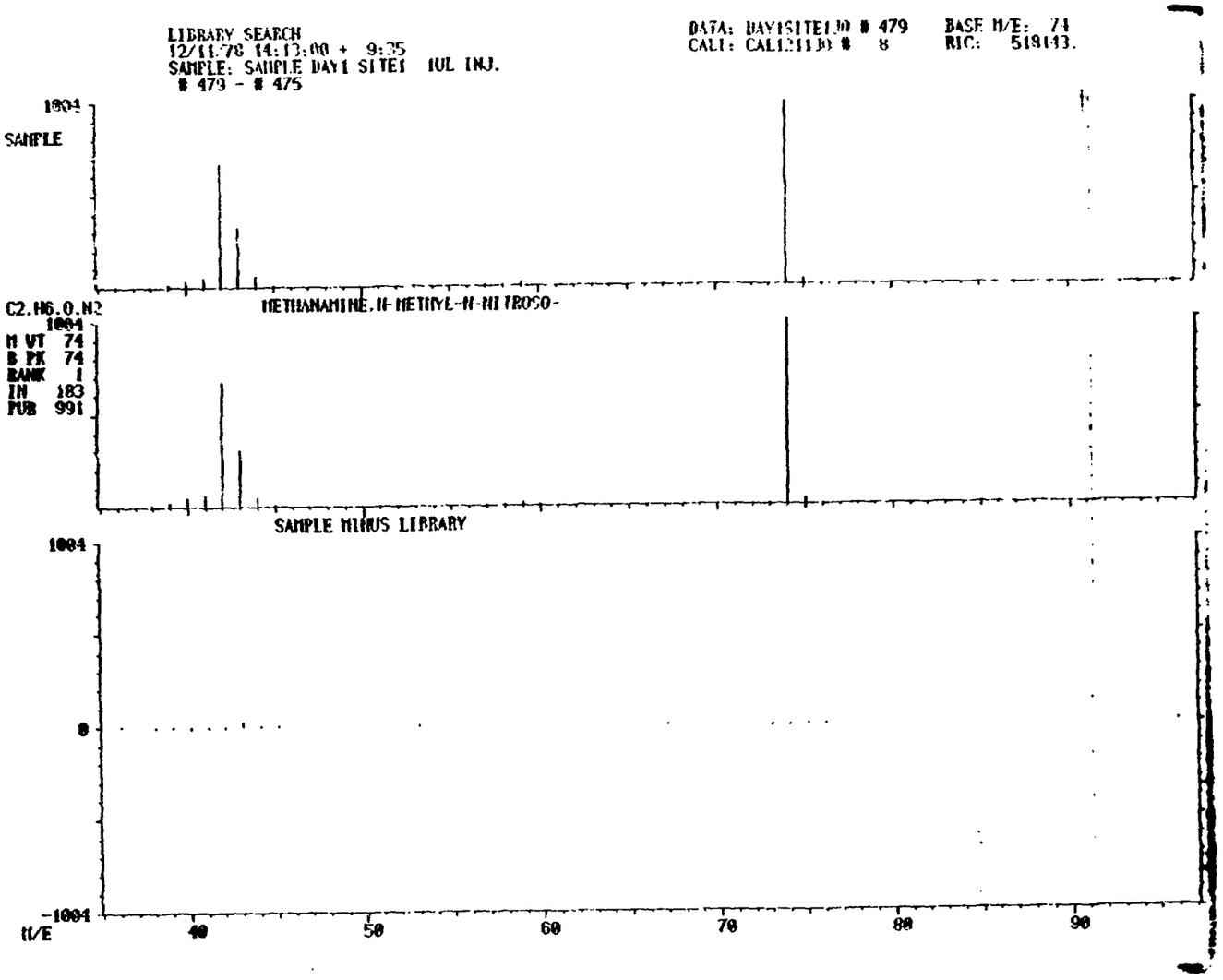


Figure 12 - Site 1, Day 1, Visual Comparison of Library DMN Spectrum and Observed Spectrum

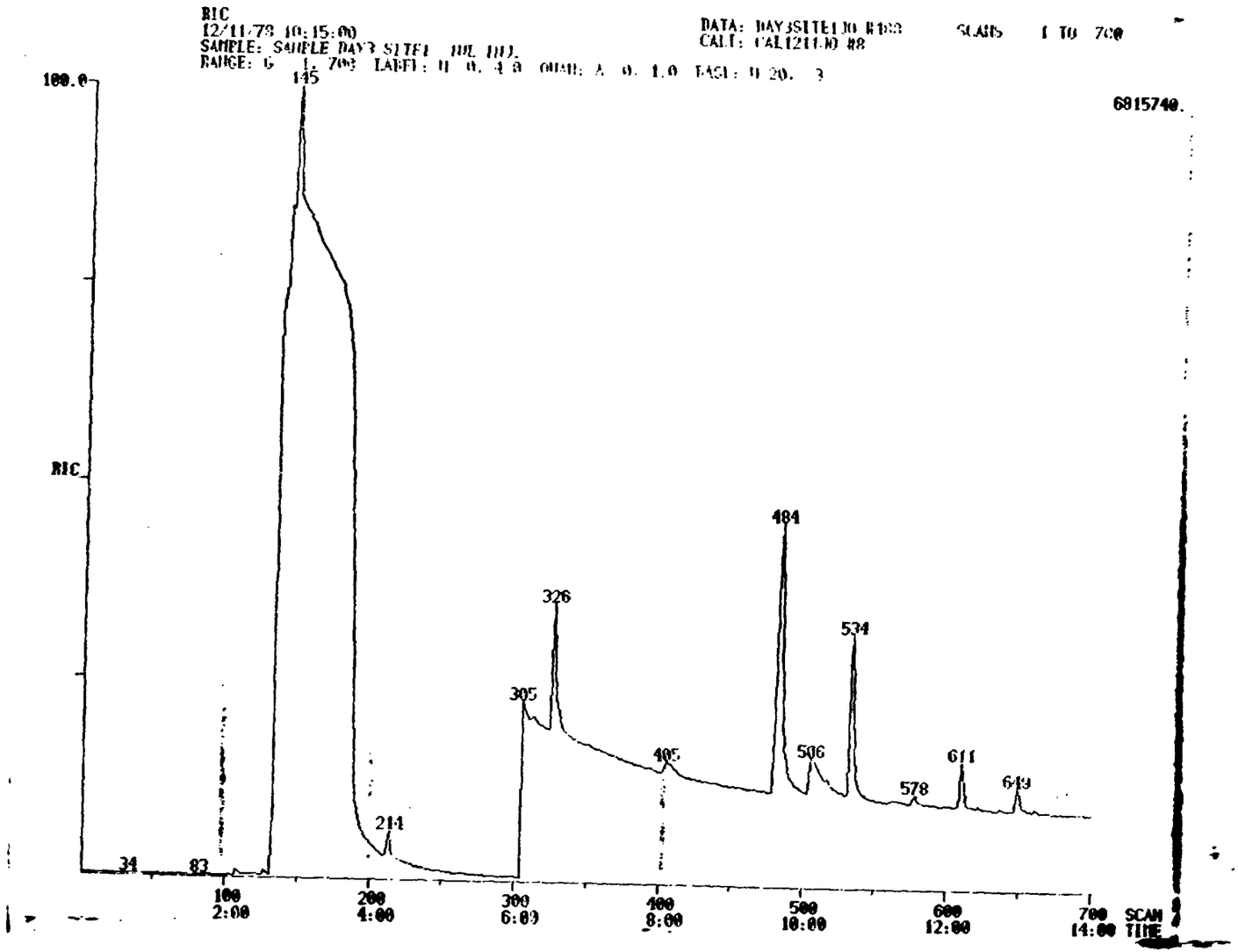


Figure 13 - Site 1, Day 3, RIC

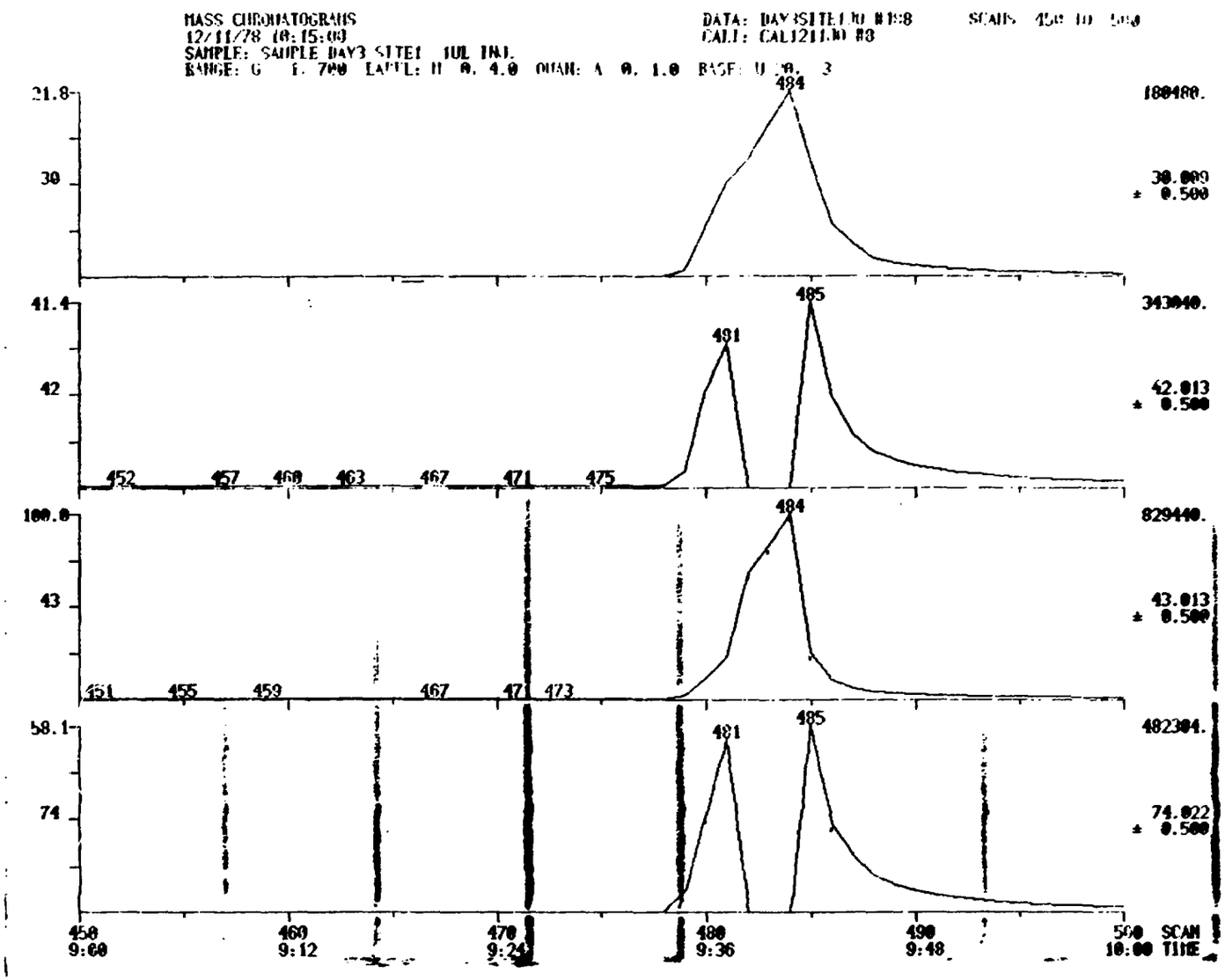


Figure 14 - Site 1, Day 3, EICP for m/e 30, 42, 43 and 74

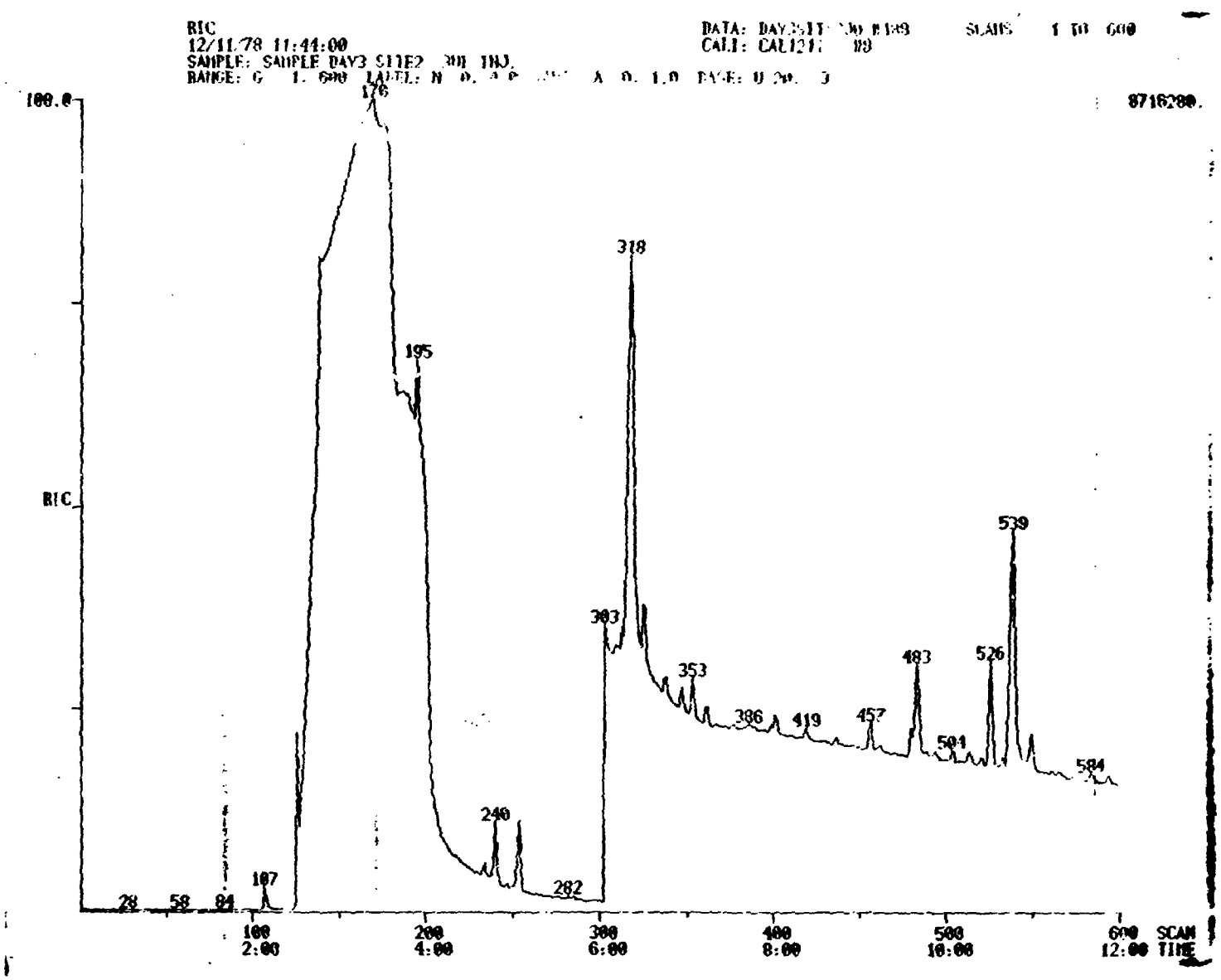


Figure 15 - Site 2, Day 3, RIC

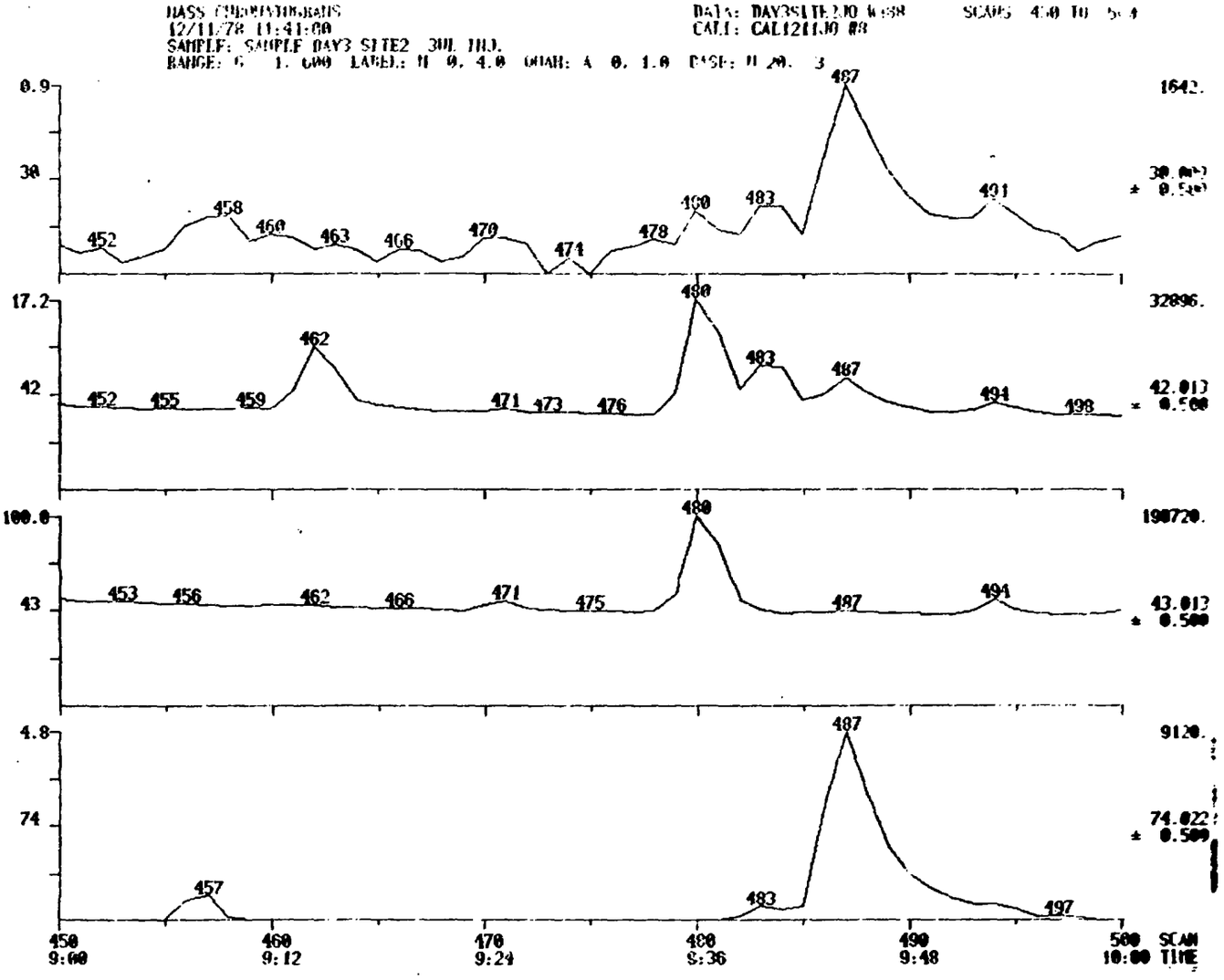


Figure 16 - Site 2, Day 3, EICP for m/e 30, 42, 43 and 74

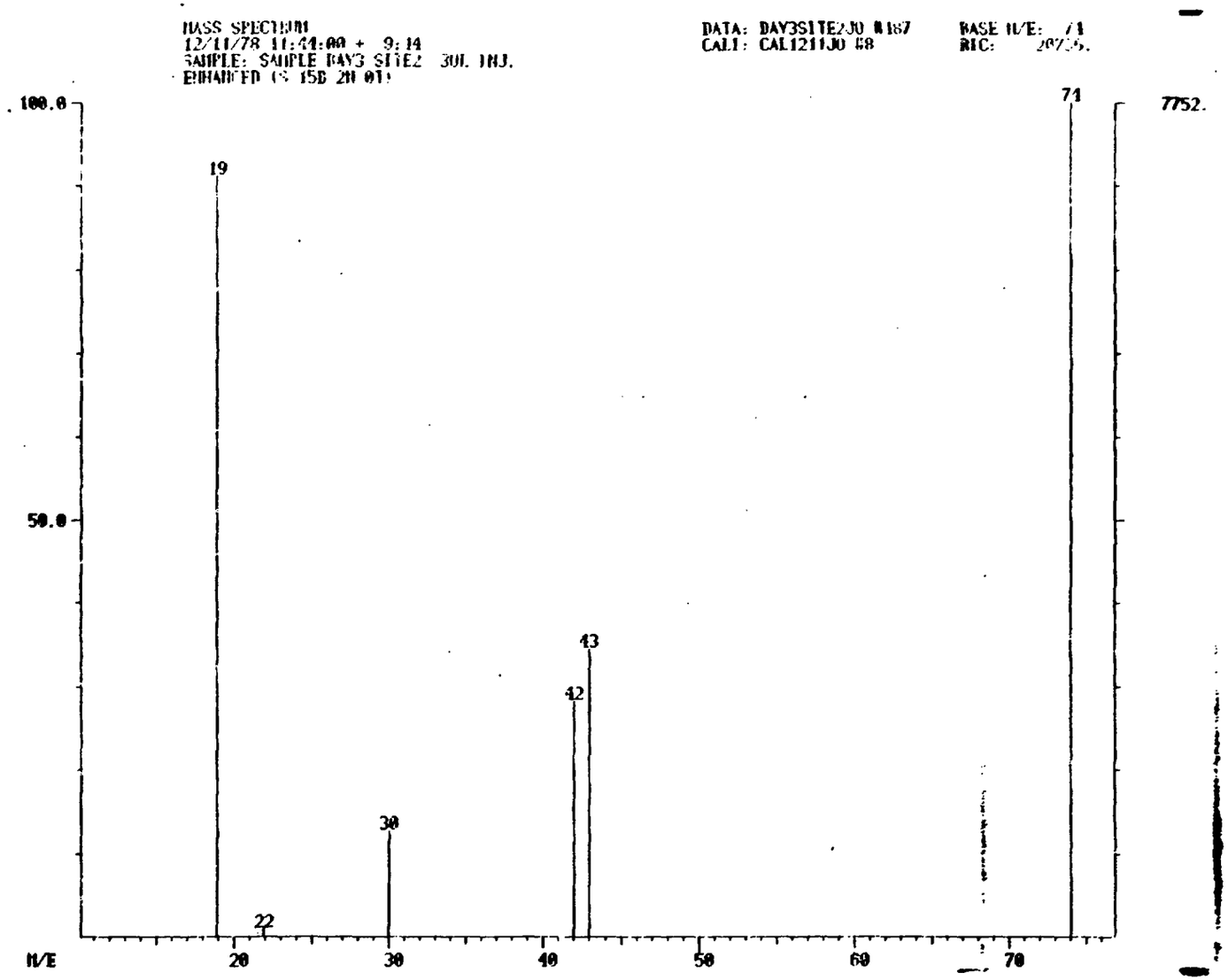


Figure 17 - Site 2, Day 3, Bar Plot, Scan 487

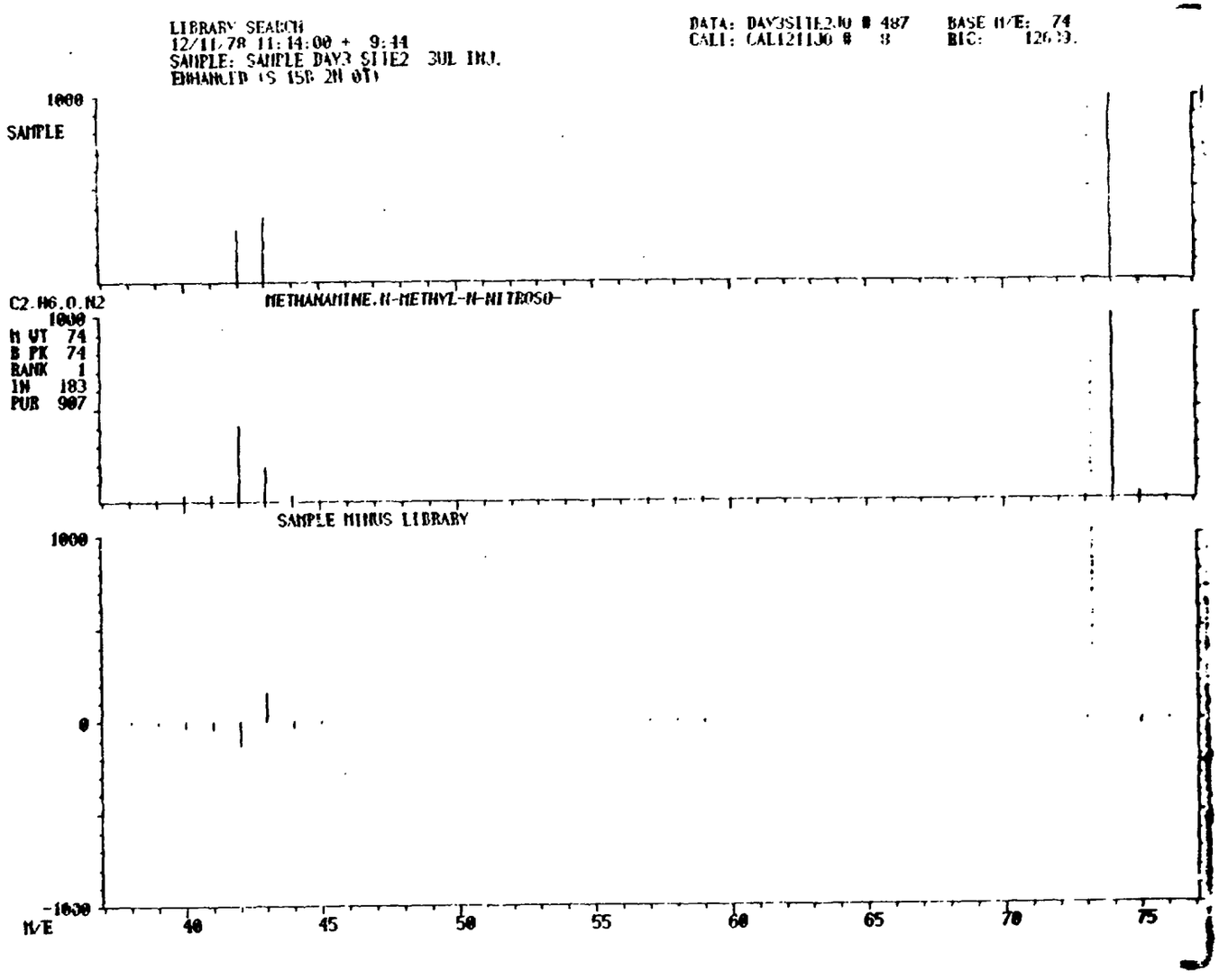


Figure 18 - Visual Comparison of Library DMN Spectrum and Observed Spectrum

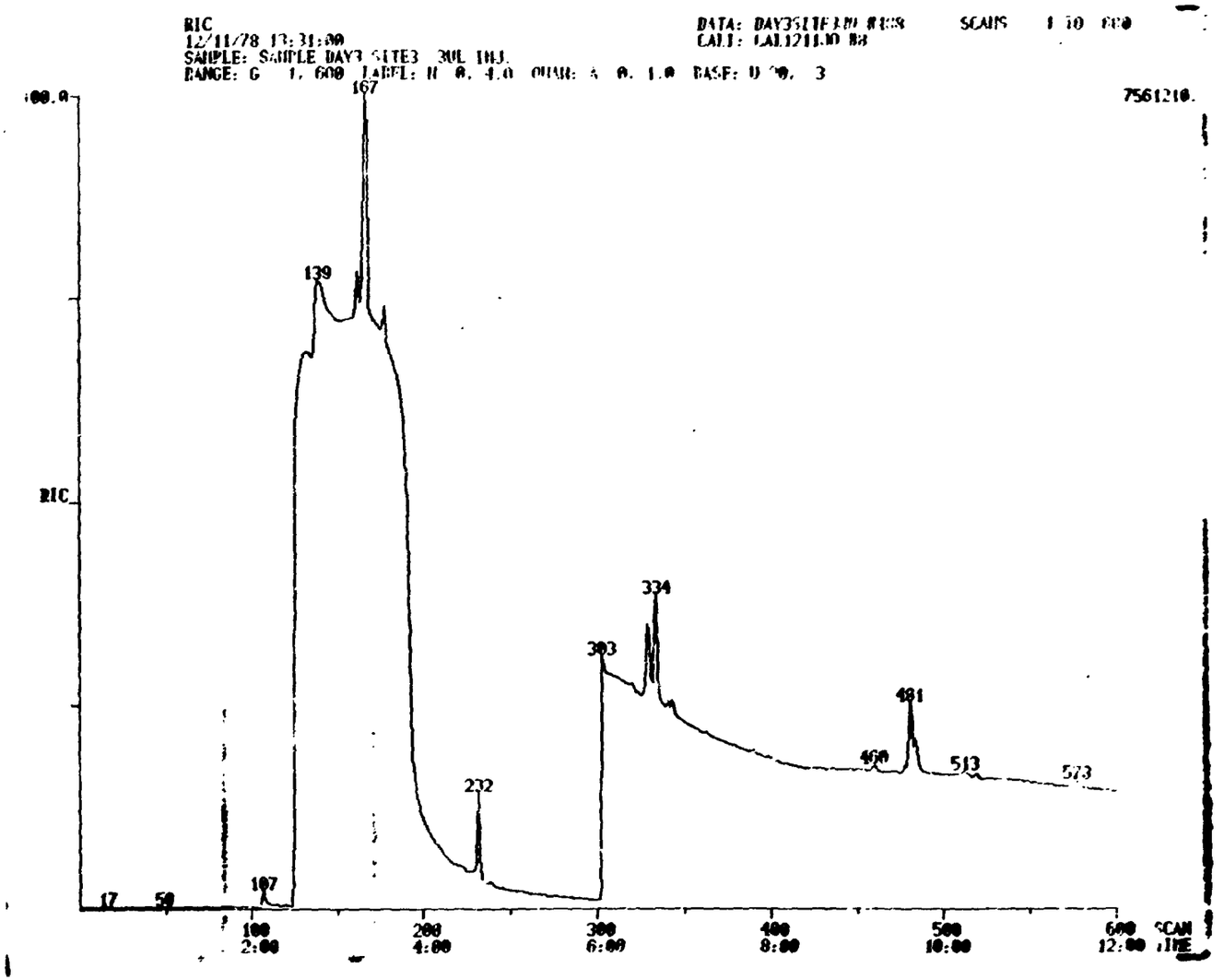


Figure 19 - Site 3, Day 3, RIC

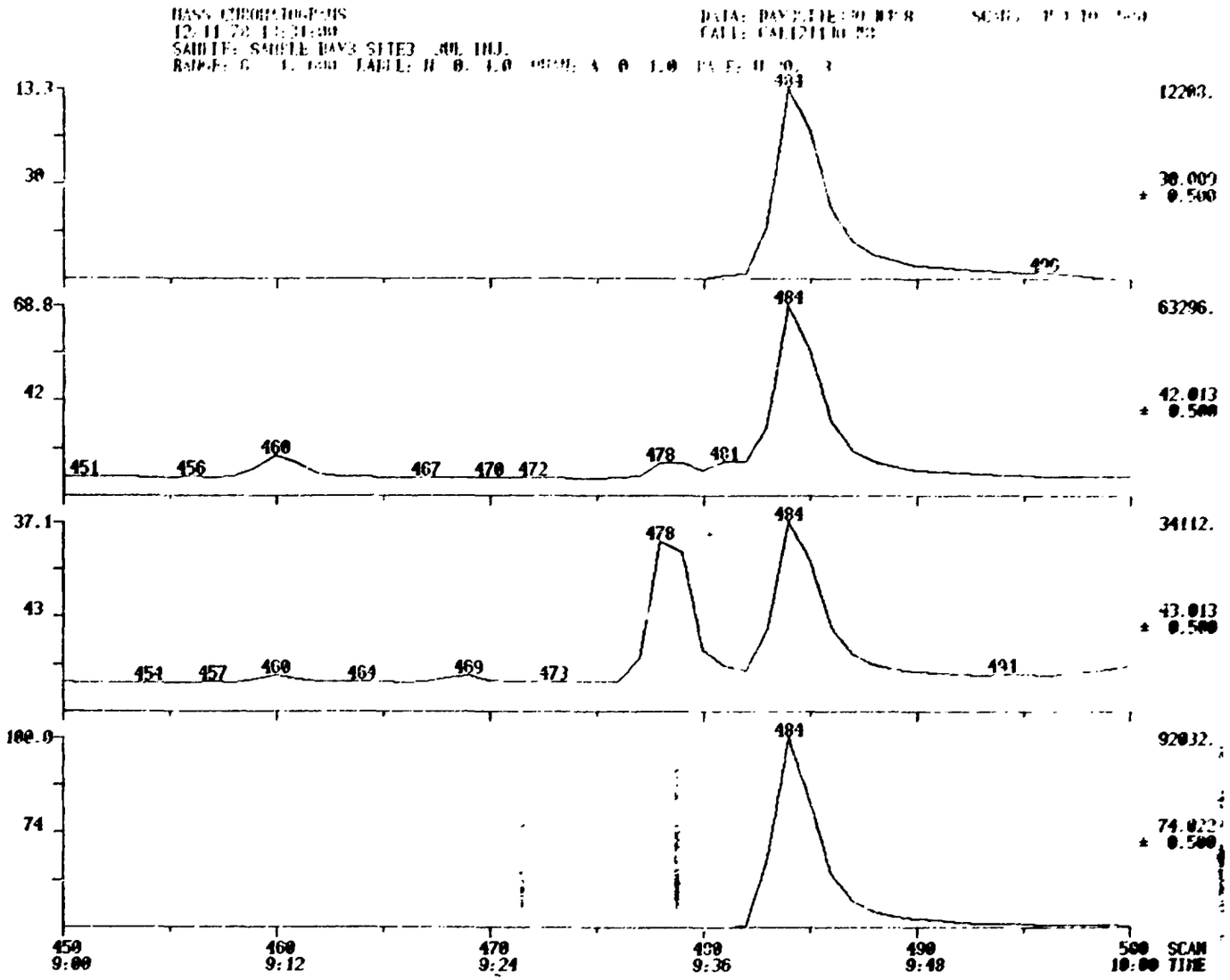


Figure 20 - Site 3, Day 3, EICP for m/e 30, 42, 43 and 74.

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